

Turning point for nuclear power

The British nuclear power programme is not alone by being in a mess, but its particular condition is a pointer to other faults in British industry — and to some general remedies.

THAT British nuclear power has fallen on bad times is well known, but not on that account especially shameful; other nuclear power programmes are also in the doldrums. But the causes of the British condition may be different from those elsewhere, and may also typify the more general sense of being moribund that pervades a large part of British industry. That is one reading of the admirable report on radioactive waste produced last week by the House of Commons Environment Committee (HMSO, £9.85), which is a level-headed and perceptive account of one part of the problem. Particular recommendations apart, the document sustains the more general belief that the perennial faults of British heavy industry are mismanagement, indecisiveness and parsimony, all veiled from public scrutiny to such an extent that rational assessment of the zigzags of policy is virtually impossible.

The immediate British discontents have their origins in the well-documented sleep-walking decision of the first postwar British government to become, first, a military nuclear power and, then, a major producer of electricity from uranium. Then, two plutonium-producing reactors and a fuel reprocessing plant were built at a site called Windscale on the coast of Cumbria (as it now is) in north-west England. In 1957, less than two years after the Queen of Great Britain and Northern Ireland had opened on the same site the first of a chain of dual-purpose reactors (for making both electricity and plutonium), the fuel in one of the original reactors caught fire, releasing radioactivity (mostly iodine) in a much more serious nuclear accident than that at Three Mile Island a decade ago. Since then, Windscale has become a persistent source of public controversy about the leakage of radioactive materials, most but not all of which are inconsequential for the health of the general public and of those working at the plant. Much of this trouble is partly explained by the antiquity of the plant, the cramped condition of the Windscale site (now called Sellafield) and the degree to which its efficient management is hampered by the large amounts of radioactivity stored there, not merely in dedicated storage tanks and other facilities but in those patches of ground that have been contaminated by past accidents on the site. Yet within the past decade, the present owner of the site, nationalized British Nuclear Fuels Ltd, has embarked on the construction of a plant for reprocessing irradiated uranium oxide fuel, eventually to service British pressurized water reactors but, more immediately, to service reactors elsewhere in the world.

Disposal

This state of affairs is one of the principal reasons why the House of Commons committee embarked on its inquiry in the first place, and why its inquiry is concerned chiefly with waste disposal. The first of the committee's recommendations, technical though it may seem, illustrates the temper of its report. Present practice at Sellafield, as elsewhere in the British nuclear industry, is to classify radioactive wastes as low-level, intermediate-level and high-level wastes; materials of the first kind are buried beneath earth in ground trenches, those of the second kind are stored in silos (but there are plans to compact them and embed them in retrievable concrete blocks) and those of the third kind

are stored in artificially cooled tanks pending incorporation into vitreous solids beginning in the 1990s. The distinction between the three categories is based on the instantaneous radioactivity of the materials, with some allowance for the separate properties of alpha and beta activity, as indeed radioactive waste materials have been classified for the past forty years in Britain. But why not, the committee asks, be more subtle and more sensitive, classifying materials according to the elemental source of their activity? For, as every schoolchild knows, there is a world of difference between the health hazard of a few microbecquerels of plutonium and of, say, strontium.

Decisions

Much in the same vein, the committee argues that circumstances have changed since the decision to build the oxide-fuel plant at Windscale was taken in 1978. In particular, the market price of natural uranium has fallen sharply, chiefly as a reaction to the general slump of interest in nuclear power, so that the economic case for reprocessing nuclear fuel (for the sake of the plutonium that might itself be used as fuel in breeder reactors) has declined. This (see p.204) appears to be the basis for such opposition as remains to French activities of this kind, and the committee asks that the British government should mount an inquiry on the subject, reconsidering the decision to build the oxide plant if the economic case for going ahead is insubstantial. The committee's doubts are reasonable enough, but they have been given prominence in its report only because of the way in which each new reprocessing plant has come to seem a potential source of further unpredicted leaks of radioactivity, any one of which might be catastrophic. This, to the nuclear industry the most damaging of the committee's recommendations, is not so much an opinion about the economic justification of the reprocessing plant (whose builders say it will be paid for by contracts already signed) but a sign of diminished confidence in the managers of the plant to operate it safely.

But have not the dangers of reprocessing been exaggerated? That is the natural defensive reaction of the Sellafield managers. So they have, and the House of Commons committee implicitly agrees, especially in its condemnation of the role of the Greenpeace organization in giving public currency to an irresponsible newspaper fairy-tale (to the effect that the plant managers were planning a controlled experiment in which one group of children would be fed radioactive materials). Nor does the committee fall into the other familiar trap of supposing that because plutonium can be used for making nuclear weapons, it is somehow a contribution to a peaceful world if countries such as Britain refrain from extracting plutonium from their nuclear fuel (and which is part of the mistaken inspiration of the Carter Anti-Proliferation Act in the United States). The committee's unwillingness to be stampeded by exaggerations gives the general sense of its argument greater force.

The committee is at its best in its discussion of just this psychological point, the degree to which general opinions of an organization (or of a committee's report) are moulded by the spirit in which they are informed of it. The Sellafield managers have been given a rotten time, over the past several years, by the way



in which each tiny release of radioactivity, not necessarily to the environment at large, has been blown up by sensation-mongers. But the committee is right to imply that the industry has helped to bring these troubles on itself by being less than wholeheartedly informative about its own problems. It is one thing to respond truthfully and fully to inquiries about the radioactivity in the environment, which the British nuclear industry now does, and another to be actively concerned to see that the general public is fully aware not merely of the vast difference between the hazards of, say, radioactive argon emitted from all nuclear power station stacks and that of plutonium dust such as may be carried into the atmosphere from reprocessing works. What the committee says the industry should be doing is to help its taxpayer constituents appreciate both the subtleties of the biological effects of radiation and the difficulty of the technical problems it faces. If there is an unusual cluster of leukaemia cases on the Cumbrian coast, the Sellafield managers have every interest to be the first to investigate. If one of the reasons why the planned vitrification of high-level waste hangs fire is the likely exposure of workpeople to volatiles in the glass melt, there is nothing to be lost by saying so.

Timorousness of this kind is long since out of fashion, even in Britain, where traditionally it is born of the notion that the infallibility of organizations increases with their size and public importance. Especially because British Nuclear Fuels still handles military plutonium, and so is able to hug secrets to itself, it is especially prone to the tendency to believe that information about its affairs should be regulated by what it senses to be the need. Its perceptions have changed over the past decade, but there is still a long way to go before it will accept that to acknowledge the problems that exist is a necessary first step towards making the nuclear industry popular. Fair play, this is a trap into which many other British organizations have fallen.

How should the British government respond (as it must) to the committee's report? The first need is to decide whether there should be yet another inquiry into the proposal to build an oxide reprocessing plant. (That in the 1970s took two years.) The simple answer is that there need not be, but that it should not be beyond the wit of sensible people to devise clear lines of authority and responsibility within the nuclear fuel company to ensure that the plant is managed well — and that it will be evident whose head should roll if it is not. Second, the government should accept the committee's relatively minor proposals that sea dumping should be set aside for the time being, until the international diplomacy as well as the technical assessment of this procedure has been carried out. Most important, the government should decide (which seems to be its intention) that it will indeed sanction the building of more nuclear power stations once the report of the Layfield inquiry is available in the summer (five years after it was set up). There is nothing so likely to sap morale as to have nothing to do. But, even more important, the government must insist that the long-term problem of waste storage is tackled with the energy that it deserves. The nuclear industry has been talking of vitrification for years, and is especially eloquent when inquiries are being held, but British development on the process has hung fire while there is still no assurance that this process is the best.

How fair is it to generalize from the sad case of the nuclear industry, the feather in Britain's self-designed cap a mere thirty years ago, to British industry as a whole? The tendency towards secretiveness, especially about difficulties, is obvious. The habit of diluting both authority and responsibility for the successful management of important projects by allowing the intervention of boards of directors in matters outside their competence is commonplace. The general failure to recognize that long-term research may have a value that goes beyond the calculable economic return (in the nuclear case, by providing the public assurance without which nothing is possible) is another general failing. So, too, are the starts and stops of government policy, which ensure that successive governments change the economic

and social climate in which important industries must find a way to prosper. British nuclear engineers now envy their French colleagues and competitors. Should not British governments also seek to emulate the sense of continuity their French counterparts provide, not simply in the nuclear field? □

Organization of research

The chairman of Britain's smallest research council deserves an answer to an honest question.

SIR Douglas Hague, the chairman of the British Economic and Social Research Council, will have lost more friends than he can have gained by his evidence to the House of Lords Select Committee on Science and Technology earlier this week (on 18 March) which is inquiring yet again into the organization of British civil science (perhaps because it had only a dusty answer from the government when it did so last, in 1981). For Hague is by reason of his office a member of the governing councils of British civil science, the Advisory Board for the Research Councils (which divides the civil science cake) in particular; there will be many who say that politeness requires people not to complain about the organizations to which they belong, as Hague did this week by saying publicly that the board has slipped into the habit of telling the research councils what to do, thus blunting the division of responsibility between them and itself.

Worse still, in the predictable opinion of some of those who will not be sending him Christmas cards in future, he endorsed a view put forward here at the end of last year that, in the interests of flexibility and of the academic research community which it exists to serve, the Science and Engineering Research Council should strive to make the Rutherford Appleton Laboratory a less conspicuous and costly part of its institutional overhead (see *Nature* 318, 587; 1985). Hague's particular anxiety is that the decision to spend £11 million on a new Cray computer for the laboratory will further enhance its permanence. In passing, he remarked on the anomaly that those who commit such sums to new projects, desirable though they may be, have rarely decided in advance which existing project they will axe in recompense.

It will be a great misfortune if this rough talk is set aside simply because it is rough. For the past decade, the British research community has been forced by an unsympathetic government to jump through a series of unwelcome and even damaging hoops, chiefly of a financial character. In many ways, the process of concentrating funds on fewer institutions and fewer people has been handled in such a way as to create diseconomies. The precipitate closing of laboratories has often, for example, meant that people who might have earned their salaries have instead been paid pensions for doing nothing. It is also probable that the attempt to drive academics into the arms of industry has been counterproductively vigorous. But none of these injustices can be welded into a case for supposing that the self-administration of science is beyond reproach. That is the essence of Hague's complaints this week.

What will the House of Lords make of it all? The case for nominating a political head of the research enterprise is as strong now as when the House of Lords made it five years ago. The greatest need is for some means of balancing the needs of civil and military research against each other. (Hague was asking, this week, why Britain has to spend 0.65 per cent of its Gross Domestic Product on public defence research and development when even France gets by on less.) There is also an urgent need to devise a mechanism for making sure that the research councils live up to their protestations, which have recently rightly tended to emphasize the importance and the need of support for academic science. Some, such as the agricultural and medical research councils, have made valiant if marginal efforts in this direction in the face of enormous difficulties, but for the system as a whole, run as it is by representatives of the research community, there is a long way to go. □

Space shuttle

No early prospects of decision on future flights

Washington

As the debate over how and whether to pay for a new space shuttle rages at the White House, the National Aeronautics and Space Administration (NASA) is trying to give shape to the agency's future in the post-Challenger era.

In testimony last week before the House Science and Technology Committee, NASA acting administrator William Graham made it clear that shuttle flights are unlikely to resume before next January. NASA estimates it will cost \$350 million over the next two years for "anomaly resolution activities".

NASA is already launched on an exhaustive safety analysis of shuttle systems. Although the presidential panel investigating the Challenger accident has yet to establish a cause, Graham conceded that changes in the solid rocket booster would probably be necessary and it would be "appropriate" to redesign the seal rings.

Even if the \$2,800 million needed for a new shuttle is found, \$750 million of which must come from the already tight 1987 budget, NASA will be able to operate only three shuttles between now and 1990. NASA plans to move slowly after shuttle flights resume, exacerbating the accumulating payload backlog. Graham hopes that commercial interests will operate expendable launch vehicles to relieve some of the burden on the shuttle, but he admitted that the current budget contains no money to help start such ventures.

Although members of the House committee are clearly anxious to allocate funds for building a new shuttle, a source close to the White House senior inter-agency group responsible for making a decision about the shuttle says no answer is likely until June, when the presidential panel makes its report. The interagency group is also wrestling with the thorny problem of which government agencies should be paying for shuttle services. The debate is being carried on by mid-level bureaucrats, but agency chiefs will soon be joining in.

Several members of the committee expressed concern that NASA might encourage potential shuttle customers to look elsewhere for launch services. But Graham confirmed that a proposal to carry the British Skynet satellites aboard the shuttle had already been terminated. With 32 launch commitments already on the books, Graham argued that NASA could not commit itself to others until the future of the shuttle was clear.

When flights do resume, priority will be

given to those carrying payloads "critical to national security priorities", followed by scientific launches with fixed launch windows, and finally all other flights specifically needing the capabilities of the shuttle. Both the Galileo mission (see below) and Ulysses, the European Space Agency (ESA) mission to explore the Sun's polar regions, have launch windows in June 1987, but Graham doubted that both could then be launched.

No matter what decisions are made about the shuttle, NASA plans to go ahead with the manned space station programme as scheduled. It is expecting to sign agreements shortly covering the parts of the space station on which Canada and Japan will work. Japan has been studying a multipurpose pressurized module, while Canada is developing a remote manipula-

tor system. But an agreement with ESA over European participation has hit a snag, and NASA administrators are working feverishly to hammer out an agreement before the end of March, when an engineering meeting will establish the space station baseline configuration.

As acting administrator, Graham has presided over NASA during its most difficult time. The agency's reputation has suffered as much of a setback as its flight schedule. The budget analyst whose July 1985 memorandum showed that NASA was aware of safety hazards in the solid rocket boosters said in a *Washington Post* article (16 March) that he had been asked to "deny the validity" of the memorandum in ensuing press enquiries. In the article, the analyst denounces NASA for perpetuating a stubborn optimism that flies in the face of its employees' conscientious warnings.

James C. Fletcher, nominated by the White House to take over as permanent administrator of NASA, is said to have had little enthusiasm for the job, and agreed to accept it only after considerable persuasion.

Joseph Palca

US space programme

Delay not an unmixed curse

Washington

WHILE it will be for Washington to decide how the US space programme will be continued in the post-Challenger era, the managers of individual projects are having to arrange that spacecraft poised for launch later in this year will survive long and still unknown delays on the ground. But uncertainty about the likely launch time may bring some unexpected benefits.

This may be the case with the Hubble Space Telescope, which was to have been launched next August. The deadline for the submission of research proposals has now been put back, which may give the Space Telescope Institute time to resolve the dispute about allocations of observing time (see *Nature* 318, 591; 1985).

The extra ground-time will also give engineers a chance to reduce the risk of "infant mortality" among the components of the telescope. This term refers to the failure of electronic parts which is common in the early weeks of space missions. The remedy is to switch on instruments before the launching of a satellite or space-probe, to "burn in" the electronic circuits.

Even so, James Welch, the NASA (National Aeronautics and Space Administration) programme director for the space telescope, says it will "not be a trivial matter" to store "this beast" on the ground until shuttle flights can be resumed. Mechanical systems designed to function in microgravity environments may not fare so well at 1 g, while gyroscopes must be spun at regular intervals to

prevent the pooling of lubricants and so as to redistribute weight. Similarly, the light-amplification assemblage of the faint-object camera, which has been designed and built by the European Space Agency, will have to be powered up every 30 days so as to maintain the internal vacuum.

A further difficulty is that career advancement is unlikely while spacecraft sit on the ground, which means that project leaders may be tempted to try their luck elsewhere. The way in which long-term space projects may suffer, even after launch, is illustrated by the Voyager missions to the outer planets, which have had six project managers since their inception more than a decade ago.

Nevertheless, the hope is that professionals who have already devoted a large part of their career to a project will not be driven away by a delay of another year or two. Torrence Johnson, head of the Galileo mission to Jupiter, says that people working of such projects have learned to cope with the frustration of delay.

Part of the Galileo team will be kept busy recalculating the trajectory of the spacecraft. The next launch window will be in June 1987. The plan is that Galileo should make ten orbits around Jupiter in a period of 20 months, but all the orbits will be different from each other so as to maximize the number of jovian moons encountered. In a celestial version of a 12-cushion billiards shot, the spacecraft will be helped on its way by the gravitational influence of successive moons.

Joseph Palca

Halley's comet

Giotto confirms reports of dumbbell-shaped nucleus

Darmstadt

THE notice DO NOT PANIC hung in one of the experimental areas here last week and, throughout a somewhat vertiginous approach by Giotto, the European Space Agency (ESA)'s spacecraft, to the core of comet Halley in the first few minutes of 14 March (GMT), and despite a loss of signal at closest approach, nobody did. And there, at last, was the nucleus. "It looks like a peanut", said one. "A potato", said another. And it is black.

It was the perfect day for an encounter. The solar wind was calm, with a speed of about 380 km s^{-1} with a steady interplanetary magnetic field of about 8 nanotesla. Earlier, there had been fears that solar activity might produce choppy electromagnetic conditions, in which case information from satellites such as the Solar Maximum Mission might have been essential to distinguish the effects of a gusty plasma environment from truly cometary phenomena.

Before the Giotto encounter, expectations were conditioned by the two Vega encounters the previous week (see *Nature* 320, 97; 1986). The Soviet chief scientist, Roald Sagdeev, had brought the news that Vega 2 had shown that the nucleus looked rather like a potato of maximum length 11 km. But there was some fear that the Vega detectors had been deceived by the variable brightness of the dust. "I'm convinced Vega saw the nucleus", said Fred Whipple, architect of the prevailing "dirty snowball" model; "there were earlier reports of a double nucleus, but the imaging people now say it's more like an irregular potato with two bright ends."

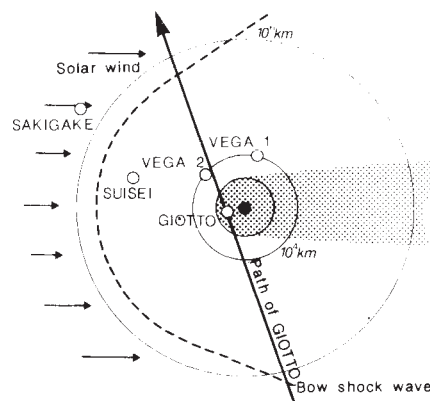
With its tenfold increase in resolution, Giotto could be expected to resolve the debate. Meanwhile, the dust specialists were perturbed but philosophical about reports from Japan of large and potentially disastrous dust particles detected by the Suisei spacecraft at the surprisingly large distance of more than 150,000 km from the nucleus (see *Nature* 320, 99; 1986).

In the event, Giotto first detected effects of the comet on its environment at a distance of 7.8 million km, with cometary ions and plasma waves becoming more and more noticeable. At this point, Giotto had entered the outer coma, a vast invisible cloud whose expansion is virtually unhindered by Halley's tiny gravity. Cometary hydrogen and oxygen atoms plus hydroxyl radicals began to be detected, the results of photodissociation of water vapour sublimed from the nucleus.

As the solar wind picks up such atoms, it is decelerated to a point (roughly corres-

ponding to a 25 per cent increase in mass loading) where a bow-shock should form, with a sudden change in flow conditions. There had been some debate whether such a shock had been detected at comet Giacobini-Zinner by the International Cometary Explorer. Pretty much on schedule, however, according to principal investigator Alan Johnstone, Giotto entered a weak bow-shock (or bow-wave) at 1.15 million km, extending along its trajectory over 0.5 million km or so. The spacecraft detected a shift in solar wind direction of about 15° , a slowing of helium ion speeds to 260 km s^{-1} and fluctuations in the magnetic field of up to 20 nanotesla.

The excitement became more noticeable with the first images from a distance



Closest approach:

Sakigake	7,000,000 km
Suisei	200,000 km
Vega 1	10,000 km
Vega 2	10,000 km
Giotto	500 km

of 750,000 km though, with resolution of 50 km or so, the nucleus was not yet visible. The atmosphere was distinctly polyglot as collaborators from many parts of the globe began to pile into the surprisingly restricted space taken up by each of the ten experimental groups. By means of a direct link to Moscow, about 30 people there were also following the encounter.

At about 0.5 million km, the presence of cometary ions was much more pronounced. Here also, cometary chemistry began to be noticeable and, as its pick-up of heavy ions increased, the speed of the solar wind decreased further. "It's terrific, it's terrific", exclaimed one experimenter. But what about the dust? So far there was none, and principal investigator Tony McDonnell was discreetly beginning to wonder whether his impact detectors had failed. To his relief, the first particle finally arrived, detected on Giotto's front shield at about 200,000 km.

At about 48,000 km, the nucleus seemed to be resolved as an unchanging feature in the camera frame. A few minutes later came the first hint of trouble as the attitude sensor indicated a slight shift in the spin axis. But by now the instruments were working overtime as Giotto approached the nucleus from behind its left shoulder, as it were, and heading towards the Sun-side, from which most of the outflow of gas and dust occurs.

As Giotto's camera followed the changing relative position, its images were relayed live, every few seconds, to the watchers on the ground. A few minutes before the closest encounter, the region of the nucleus began rapidly to dominate the screen, and mottled features became apparent. It was a frankly emotional moment; the quiet voice of the scientific commentary began to shake with excitement and the assembly broke into applause. At about 5,000 km, the magnetometers saw the magnetic field vanish — Giotto had penetrated the ionopause where electrical currents carried by electrons and ions cancel the ambient field.

Almost to the second of closest encounter, at a distance of 605 km, the images ceased and the telemetry link at the 64-m Parkes radiotelescope in Australia lost contact with the spacecraft. Tony McDonnell later said, "we seemed to hit a wall of dust a few minutes before the encounter". The impact rate had then gradually increased from the already high rate of 120 per second to the point where the spacecraft suddenly went out of alignment. But in the belief that the spinning spacecraft had been knocked into a wobbling precession, damping manoeuvres were carried out and contact re-established after 30 min.

And the nucleus? According to the camera principal investigator Horst Uwe Keller, first indications are that it is 15 km long and at least 4 km wide, perhaps shaped like an indented potato or peanut shell. The loss of contact meant that only one side of the nucleus was seen, so that an accurate evaluation of the volume will not be possible, nor will the variation of the reflecting properties of nuclear material with Sun angle be fully known.

Nevertheless, surface features have been seen with a best resolution of 70 m. The nucleus is "blacker than coal", according to Keller, "similar to the lowest albedos yet seen in the Solar System". He says that the surface must be at least warm enough to melt ice. Fred Whipple believes that such an appearance could arise from ice embedded with a great deal of dust including carbon or organic material.

Perhaps the major surprise of the encounter, according to chief scientist R. Reinhard, was the great inhomogeneity of dust emission. To a much greater extent than expected, the dust outflow is concentrated in jets and there are indications of nozzle-like features on the surface. A

most important task will now be to match *in situ* observations of jets with those provided by the International Halley Watch network.

Analysis of mass spectrometry also proved exciting. "When we moved into the coma, we were overwhelmed by the richness of the neutral and ion spectra", said principal investigator D. Krankowski. The major ions turned out to be those associated with water vapour, H_3O^+ and H_2O^+ , with a sharp increase in density to a plateau of about 10^3 per cubic centimetre at about 30,000 km; among the neutrals, H_2O reached 10^8 cm^{-3} at about 3,000 km. The total water outflow was estimated to be about 2×10^{30} molecules per second. Compounds of carbon, nitrogen and oxygen were also seen by optical spectrometry. C_2 was found to be more abundant than CN , the latter and CO^+ increasing from a distance of 150,000 km with a sharp rise in CO^+ just before approach. Metal ions were also seen, though as H. Balsinger indicated, if the nucleus is a potato it is not a salty one; little sodium was seen.

The blackness of the nucleus may be linked to another surprise reported by Tony McDonnell — the presence of an unexpected population of dust with masses as small as 10^{-17} grams. As Vega dust scientist J.S. Simpson remarked, most models predict a fall-off in dust masses below 10^{-12} grams, but Vega and Giotto have indicated substantial numbers of smaller particles. It had been thought that such particles would have been expelled from the Solar System by radiation pressure before they could be incorporated into comets. The efficiency of such processes may now have to be re-evaluated in view of the photo-properties of the nucleus discovered by Giotto.

The density of each dust particle seems to have been less than that of water, indicating a porous structure, possibly with a solid core covered with an icy mantle.

Comparing the Giotto and Vega missions, Sagdeev said that the gas, dust and particle measurements showed very good correspondence while highlighting the variability of the neighbourhood of Halley's nucleus. The Giotto measurements of *in situ* chemistry should be matched with Vega's remote sensing spectrometers, whose resolution might permit investigation even closer to the nucleus than Giotto.

"The science was really great", he said. "After many sleepless nights, we have to part with the comet. We feel real nostalgia now which will last beyond Halley's next apparition."

Meanwhile, Giotto is still working, although with possible damage to the camera and altitude control. It is hoped to adjust its orbit, enabling it to use the Earth as a gravitational sling in 1990, perhaps to encounter comet Grigg-Skjellerup in 1992.

Philip Campbell

Book conservation

Red faces over US pilot plant

Washington

AN explosion and fire have raised design and safety questions about a book conservation process developed by the Library of Congress (LC) that supposedly presented "absolutely no safety risks to personnel or books". The process was designed to make book paper less acidic.

The explosion occurred at a pilot facility operated by Northrop Services Inc., under contract to the National Aeronautics and Space Administration (NASA) at the Goddard Space Flight Center in Greenbelt, Maryland. The facility was designed to support start-up of a \$11.5 million Mass Deacidification Facility for LC to be constructed at Fort Detrick, Maryland.

What started as a small leak of the chemical used in the deacidification process in early December ultimately resulted in the "controlled disassembly" of the entire system by a demolition team on 21 February.

Book deterioration caused by the high acid content of paper made from wood pulp is a serious problem for all libraries. To decrease the acid content, LC developed and patented a vapour phase process using diethyl zinc (DEZ). The process works by placing books in a vacuum chamber at 20 torr, removing most of the moisture from the books, then injecting gaseous DEZ into the chamber. The DEZ reacts with sulphuric acid in the paper, forming zinc sulphate and water, and the water then reacts with additional DEZ to form zinc oxide in the paper fibres. This provides an alkaline reserve against further acid degradation. Zinc carbonate, produced by adding carbon dioxide to the reaction, also buffers the paper, as well as providing some measure of protection against photodegradation of cellulose.

While the DEZ process has advantages over other liquid processes that have been developed, DEZ is a difficult material to work with. It is pyrophoric in its liquid state at room temperature, burning on contact with air and explosively decomposing on contact with water.

On 5 December, with the book chamber in the pilot facility empty, the system was put through its operational cycle. At the point where all DEZ should have either been recovered or vented, the door to the chamber was allowed to open. The technician on duty reports seeing approximately one litre of liquid DEZ spill out of the chamber onto the ground. Automatic sprinklers extinguished the resulting fire in 8 seconds, and only minor damage resulted, but project engineers immediately shut-down the system while they attempted to discover why liquid DEZ had formed in what was supposed to be a vapour phase of the process. The best

guess, according to James Robinson, acting associate director for institutional management at Goddard and chairman of the board of inquiry into the accident, is that some pipes in the gas delivery system had cooled to the point where DEZ would condense, even at low pressure.

When NASA and Northrop engineers returned to the facility early last month, they discovered a positive pressure in the recovery leg of the system. Assuming that additional DEZ or ethane, a reaction by-product, still remained in some of the process piping, they attempted to vent the remaining gas. During this process, a "significant" explosion occurred, destroying valves, pipes and instrumentation, and compromising the ability to "safe" the system with nitrogen gas.

NASA and Northrop engineers were unable to tell whether any DEZ remained in the process piping, and, if it did, how to remove it safely. Robinson says project engineers considered punching a hole in the remaining pipes from a distance with a rifle bullet, but rejected single-point venting because all remaining DEZ might not escape. NASA and Northrop ultimately concluded that the only way to make the facility safe was to destroy it. An army demolition team set off ten shaped charges on 21 February, "disassembling" the system. The DEZ released at this point burned off in 30 seconds, but this fire spread to the wooden structure housing the system, which burned for nearly an hour. Damage was estimated at \$30,000.

Both NASA and LC are satisfied that adequate safety procedures were followed in the design of the test facility. If anything, NASA and LC officials say the nature of DEZ prompted excessive safety concerns about its handling.

But safety issues have been raised in the past about how NASA and Northrop conducted the deacidification testing. In 1982, a Northrop safety engineer was fired for insubordination after bypassing his own superiors and reporting safety concerns about the project directly to NASA.

While Robinson would not rule out the possibility that safety issues may have been neglected, he contends "the safety review was adequate... but the fact is that we have had two accidents in three months".

LC has delayed opening of bids for construction of the Mass Deacidification Facility for at least a month until the board of inquiry makes its report.

Peter Sparks, director of preservation for LC, says the pilot system was designed to point out design problems. "We learned a lot from this, but it wasn't quite the way we wanted to learn it."

Joseph Palca

Nuclear reprocessing

France avoids British problem

Do the French do their reprocessing better than the British? The answer seems to be yes, at least by the criteria of leakage from and accidents at the principal French reprocessing plant at Cap de la Hague, on the English Channel coast near Cherbourg. Even opponents of the French nuclear programme admit that leakage is no longer a problem; environmentalists have turned their attention to other matters.

Even so, La Hague may be storing up problems for the future, for much of the low and medium level waste that British Nuclear Fuels discharges into the Irish Sea from Sellafield is stored on the land in Brittany. In short, the long-term nuclear waste problem has not been solved.

Nevertheless, French emissions of radioactivity from La Hague, the principal reprocessing plant, are low. The independently supported claim is that La Hague discharges into the English Channel 220 times less alpha emission, and seven times less beta emission, than does Sellafield into the Irish Sea. And despite the large amounts of waste stored on land, the average radiation exposure of a La Hague worker is 2.5 times less than that of workers at Sellafield.

But not everything has been rosy. La Hague suffered a series of setbacks in the 1970s. As in Britain, French nuclear power began with Magnox gas-cooled reactors, and early reprocessing of this fuel was very inefficient. Process pipes shielded in concrete had constantly to be broken open, exposing workers to high levels of Purex process (in which wastes are precipitated from a solution of the spent fuel in nitric acid). In the late 1970s, throughput at the plant was only a quarter of design capacity. La Hague also suffered a serious transformer fire which closed down pumps in the plant and raised questions about safety and the redundancy of equipment. (There was no second transformer to bring on line.)

Now, most of the limited amount of French Magnox reprocessing has been transferred to a new plant at Marcoule on the Rhone, and La Hague is concentrating on reprocessing the more highly irradiated oxide fuel from the huge French collection of pressurized water reactors (PWRs). This is the connection in which La Hague is proving efficient and relatively clean.

According to COGEMA, the French nuclear fuels company for which reprocessing is a third of turnover, La Hague is currently responsible for reprocessing 80 per cent of the world's reprocessed oxide fuel (most of it French but some from Japan, Belgium, West Germany and elsewhere). COGEMA has a clear commercial interest in proving itself better than Sellafield, with which it will be competing

directly in the 1990s, when the Sellafield "THORP" plant is due to be on stream.

COGEMA is a relatively secretive organization. French civil and military nuclear institutions overlap, and access to the La Hague plant is tightly controlled. But the doors have been opened, notably to a team of physicists, including nuclear opponents and headed by Professor Jacques Castaing, which reported on La Hague a couple of years ago. Moreover, many members of the antinuclear French trades union CFDT are workers at the plant, and are not slow to report accidents to their union. So although independent information on La Hague is not easy to come by, it is available. The general

impression is that La Hague does not leak.

This is why the French environmentalists' attack on reprocessing is directed at the prospect of a "plutonium economy" and at the costs of reprocessing. The company claims that costs have actually fallen by some five per cent over the past year because of the smooth operation of its UP II oxide reprocessing stream, which handled 418 tonnes of fuel last year, 18 tonnes (or nearly five per cent) more than the plant's targeted 400 tonnes. Reprocessing costs would now be some FF5,000–6,000 per kg of spent oxide fuel (£500–600) according to COGEMA, but its opponents say this figure involves some sleight of hand over discount accounting, and that the true costs will turn out to be twice as much. Indeed, the critics say that the electricity utility EDF would not be able to afford the true costs. **Robert Walgate**

Fusion in Japan

Plans for second major machine

Tokyo

A NEW institute for basic studies in nuclear fusion seems likely to be built in Japan quite separately from that supporting the massive JT-60 tokamak project. An important step towards this goal was taken last week with the submission of an official recommendation from the Ministry of Education, Culture and Science (MESC)'s Science Council.

The institute is likely to be centred on the Nagoya University Institute of Plasma Physics, the largest university basic physics research facility, which has for some years been planning to move from the Nagoya University campus to more spacious grounds at Toki, in nearby Gifu prefecture. Parts of the land reserved for the move have already been bought with funds supplied by MESC.

Now that support has been gained from the Science Council (a twenty-seven member academic advisory board appointed by the ministry), MESC will spend some time considering whether an expanded institute should be built. A special committee will shortly be set up by the ministry.

The Institute of Plasma Physics has been Japan's chief plasma research institute for 25 years: it is an open institute at which scientists and engineers from all over Japan may come to do research and has helped give birth to several other large facilities, most notably Osaka University's laser fusion institute. After the move it could well undergo a change in status to that of a "national joint-use research institute". Such industries, like the High-Energy Physics Research Institute at Tsukuba and the Institute of Space and Astronautical Science in Tokyo, are quite independent and have been able to obtain research facilities that are often superior to those at universities. Conversion to

"national" status would almost certainly mean that Kyoto University's Heliotron group, as well as plasma physics groups elsewhere, would be incorporated into the institute.

The new institute, whatever its status, is to have a helical conductor system as its main facility, rather than a tokamak. Japan's giant JT-60 tokamak — on the same scale as the Joint European Torus at Culham (United Kingdom) and the fusion test reactor at Princeton (United States) — is run quite separately by the Science and Technology Agency.

At JT-60, expansion is also expected following the submission of a recommendation from the Atomic Energy Commission in the middle of February. Although there are tokamaks in the universities, it is overall variety rather than scale that has been aimed at in fusion machines; in laboratories around the country can be found heliotrons, stellarators and bumpy torus machines, open-ended "magnetic bottles", reversed-field pinch torus machines and compact toroids, including spheromaks.

A conceptual design for the new helical conductor system will be worked on by a committee in the Institute of Plasma Physics. Its nearest relatives are likely to be the Advanced Toroidal Facility under construction at Oak Ridge in the United States and the "Deep Well" project at Wendelstein in West Germany.

If all goes well, purchase of the land and clearing of the site will be completed in the next fiscal year. How quickly the new institute will then be built will depend on how quickly a consensus on its form can be reached by MESC among Japan's fusion research community — and on how quickly funds can be won from the Ministry of Finance. **Alun Anderson**

US acid rain

Academy finds causal connection

Washington

WITH a fine sense of timing, the National Academy of Sciences last week released the latest of its contributions to the study of acid rain. The academy's study*, which provides the most conclusive evidence likely to be available for some time that emissions of sulphur dioxide from power stations acidify lakes and kill fish, comes the week before Canadian Prime Minister Brian Mulroney arrives in Washington for a summit meeting with President Reagan. Acid rain is at the top of the agenda.

The academy's study, chaired by James Gibson of Colorado State University, looks at time trends and regional variations in anthropogenic sulphur dioxide and nitrogen oxide emissions over the United States, reexamining original data to ensure consistency where necessary, and relates these to effects in the atmosphere and on the ground. It concludes, on the basis of consistency in the association and demonstration of intermediate steps, that in eastern North America a causal relationship exists between sulphur dioxide emissions and presence of sulphate aerosol, reduced visibility and wet deposition of sulphate. Regional changes in sulphate in streams are consistent with these changes, and so is acidification of lakes (assessed over time by diatom remains). Because "the observed acidification cannot be explained by other known disturbances . . . acid deposition is the most probable causal agent".

The clearest instances of environmental effects of acid rain were found in the Adirondack lakes of New York, although there were marked variations in the sensitivity of different lakes. Gibson characterized the results last week as establishing a "very significant linkage" between emissions and acidification. Furthermore, fish populations appear to decline concurrently with acidification. But geographically widespread reductions in tree-ring width and increased mortality of red spruce in the eastern United States, occurring concurrently with climatic anomalies and with high rates of acid deposition, could both be laid unambiguously at the door of acid rain. Members of the academy's committee said last week that the causes of tree mortality should be the next major acid rain research project. Because of poor time-trend data, few conclusions could be reached about nitrogen oxides.

Environmental groups, which have long been convinced of the damage caused by sulphur dioxide, saw the study as further confirmation of what they already knew; the basic hypothesis that sulphur dioxide emissions have caused acidification of lakes has been accepted by several academy and other studies since 1981. The

weight of the study would seem to lie in its demonstration that such effects are large in relation to natural processes and dominate them statistically. But however elegant and persuasive, it is unlikely to persuade the Reagan administration to accept immediate measures to reduce US emissions, which have begun to rise again and which under present policies are likely to go on rising until at least 2010, according to projections by the Environmental Protection Agency.

Fears about the federal budget deficit, which have reached a new pitch because of

unfavourable reaction on Capitol Hill to President Reagan's budget proposals for 1987, make large federal expenditures on control technologies difficult to contemplate, despite the apparent emergence of a new bipartisan consensus favouring control measures in Congress. The discussion with Mulroney this week is likely instead to focus on the recent recommendation of two US/Canadian special envoys that \$5,000 million be spent on demonstration projects to develop cheaper ways of burning coal cleanly. The sum currently proposed for this purpose in the United States in fiscal year 1987 is \$400 million.

Tim Beardsley

*Acid deposition: long-term trends, National Academy Press, 1986.

Soviet spacecraft

Space station takes X-ray satellite

A JOINT Soviet/West European study of X rays in space, originally planned for a Salyut-type spacecraft, will now be carried out aboard the new Mir space station, according to unofficial reports from Moscow. This could cause some rethinking of the project, if, as the Soviet media suggest, Mir, after a running-in period, will be permanently manned. For, in order to ensure the accurate pointing which the experiments demand, it was originally planned to carry out the study during a period when the host station was unmanned.

The joint project, known to the Soviet side as "Rentgen" and to the West Europeans as HEXE (High Energy X-ray Experiment), was originally scheduled for April 1986, but there was some delay in the construction of the instruments. Two pieces of apparatus, designed and made by the Max Planck Institute for extraterrestrial physics at Garching (West Germany), have already been delivered to Moscow, but are apparently still causing problems. The software for the project is also still being worked out, and it now seems doubtful that the August launch date can be met.

The Soviet space planners have not yet said when Mir is likely to be ready for full occupation (cosmonauts Leonid Kizim and Vladimir Solov'ev are at present activating the life-support systems and preparing it for habitation). Some 30 cosmonauts are believed to be in training for service aboard Mir, most of whom will be involved in research and "technological production". (The operation of the station itself will be virtually automatic.) Whether there will be any intervals between "shifts" in which HEXE could be deployed as first planned is doubtful.

The HEXE experiments, designed by teams from the Universities of Utrecht, Birmingham and Tübingen and from the European Space Agency, as well as from the Max Planck Institute, are intended to

study the X-ray stars of the Galaxy, the remnants of supernovae and the nuclei of other galaxies. This will require extremely accurate pointing and minimal perturbation of the station during the experiments. Hence the original stipulation that Salyut should be in its unmanned mode. The greater mass of Mir might, however, make it possible to attain the accuracy needed of HEXE, even if one or two people were on board the station. Vera Rich

German gigaflops

Hamburg

THE West German government is to support a project for building a huge supercomputer designed around more than a thousand microprocessors. This was announced last week by Heinz Riesenhuber, the federal minister for research and technology, who said that Krupp Atlas Elektronik GmbH of Bremen and Stollmann GmbH of Hamburg will be joining with the existing Gesellschaft für mathematische Datenverarbeitung (GMD) with the intention of completing the prototype of the new computer by the end of 1988.

Riesenhuber's ministry, BMFT, has already appropriated DM27 million for the project, which will be carried out by a newly formed company based in Bonn. The total cost is estimated at DM130 million. The designers are aiming at a machine capable of 1,000 million operations a second, known as one gigaflop. Much of the incentive for the development has come from GMD, which has had some success in the development of programs and programming languages for computers based on parallel architecture.

The new machine will probably be used for the simulation of large-scale phenomena, among which Riesenhuber mentioned combustion processes and wind-tunnel experiments.

Jürgen Neffe

Data protection

Swedish research under wraps

Stockholm

SWEDEN'S Data Inspectorate has caused consternation among sociological researchers by ordering the removal of all personal identification from the records of a research project run by the Institute of Sociology at the University of Stockholm, and completed at the end of last year. The project, known as *Metropolit*, consisted of the longitudinal study of 15,000 people born in Stockholm in 1952, and had cost Skr7 million (roughly £700,000) over two decades. The university has until 1 May to comply with the inspectorate's ruling.

One consequence of the ruling will be that sociologists will not be able to add to the data now on file, either now or in the future. Indeed, even the potential value of the data so far gathered for control purposes is threatened by the mounting demand, which Swedish liberal newspapers are supporting, that the files should be scrapped completely.

This development may compromise the needs of sociologists and medical researchers elsewhere in Europe for access to the individual records of research projects. In the late 1970s, an attempt to win government approval for such access, accompanied by assurances of confidentiality, was mounted within the framework of the European Science Foundation, but not in the end pursued.

This turn of events is also an interesting pointer to the changed attitude of Swedish society towards the collection of confidential data about individuals. In 1966, Dr Carl Gunnar Jansson, the head of the *Metropolit* project, was able to recruit members of his study cohort by distributing study forms to teenagers in school classrooms; social surveys were a routine part of Swedish life in which openness was considered an unavoidable virtue.

At the outset of the project, the teenagers in the sample were told in general terms of the objectives. No direct approach was made to their parents, but Jansson says that there was enough information in the general press for them to know the project's aims. To the information provided by the members of the sample were added data on educational attainment, physical and mental health and infringements of the law derived from government records themselves regarded, in 1966, as "open".

Jansson's problems began only in the mid-1970s, with mounting public concern about the confidentiality of data stored in computers. Although the Swedish Data Protection Act, which came into effect in 1974, allowed existing research projects to continue, Jansson was told in 1976 that no further data could be added to the files without the informed consent of the sub-

jects, then in their twenties. Until the Data Inspectorate reversed that ruling in 1978, the result was a two-year hiatus in the addition of data to the files, on the grounds that consent would surely be withheld by those in the 15,000-strong cohort with criminal or other unflattering records, so biasing the sample.

Although *Metropolit* has now formally ended, Jansson had sought to make the files accessible to future researchers by depositing a key to the identity of the members of the sample in the national archives. Opposition to this proposal was stimulated by an enterprising journalist of *Dagens Nyheter* (which, ironically, was a strong supporter of the survey when it began). The result has been a general outcry against "Big Brother": 5,000 of the original sample of 15,000 have either asked that their names be withdrawn or that they should have access to their records.

The university will have three weeks in which to appeal against the new ruling

once it has been promulgated officially next month, but Jansson, now lecturing in the United States, is unsure whether he will follow this course for fear that an appeal would subject the *Metropolit* researchers (of whom his wife is one) to further stress. The appearances suggest that the Data Inspectorate will extend the basis of its new ruling to other projects, having also ruled that personal identification should be removed from a follow-up study of women who have had abortions.

Meanwhile, Swedish researchers needing to compile files of personal information may be tempted to follow what is called the "Evajo" option, named after Eva Johansson who, in the mid-1970s, carried out a study of the development and social adaptation of the children of former juvenile delinquents. Considering it unethical to interview the children for fear of the psychological damage that would be caused, but denied permission by the Data Inspectorate to compile a computer file from government records, she took her study outside the scope of the Data Protection Act by assembling the data manually, on index cards. **Vera Rich**

Pesticide toxicity

US Congress acts on compromise

Washington

LAGGARD federal safety testing of pesticides will be given an \$80 million boost from industry user fees, but also a strict new timetable, if Congress passes legislation put forward by industry and environmental groups. The proposals are meant by both sides in a long-running dispute over pesticide safety to ensure that testing is speeded and that efforts are concentrated on the chemicals of greatest concern.

Many of the 600 or more active ingredients in pesticides registered before 1978 were tested during the 1950s and 1960s, when requirements were less demanding. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was amended in 1972 to require the Environmental Protection Agency (EPA) to demand comprehensive industry health and safety studies, but in the intervening fourteen years, only six ingredients have been fully tested and approved; for 475, even the data needed have not been specified.

Under the amendments to FIFRA now proposed, all the unapproved active ingredients would have to be reregistered according to a strict timetable, with industry paying \$150,000 to EPA for each material considered. Pesticides previously registered with false or invalid data would be banned. "Special reviews", undertaken when a substance fails a potentially significant toxicity or carcinogenicity test, would be limited to one year's duration; testing of all pesticides on the market would have to be completed in seven years.

Besides the active ingredients, the bill would for the first time require EPA to test thoroughly so-called "inert ingredients", which include all those that do not actually kill the pest but that contain several substances (such as benzene and cadmium compounds) not normally thought of as inert. Because there are 2,000 inert ingredients in use, EPA would each year draw up a priority list of those most in need of more safety data, data which would have to be supplied within four years. The effect of this provision, according to Nancy Drabble of Public Citizen's Congress Watch, would be to put pressure on manufacturers to use those inert ingredients that are most likely to be safe. Other provisions would improve public access to information.

The new compromise has been hammered out over the past year by 41 environmental and consumer groups under the banner of the Campaign for Pesticide Reform and 92 agricultural companies represented by the National Agricultural Chemicals Association. A bill was introduced in both houses of Congress last week, and given the broad support is thought to have a good chance of passing. But the American Farm Bureau, representing the users of the chemicals, has still not put its name to the bill. It wants to see a clause to protect farmers from lawsuits arising from approved pesticide use, and it has considerable clout in Congress. Negotiations are said to be at a delicate stage.

Tim Beardsley

West German universities

Shortening of courses urged

Hamburg

A DRASTIC shortening of university courses has been advocated by the Wissenschaftsrat, the standing committee of scientists and delegates from the federal and regional governments which functions as West Germany's senior science advisory board. In a 130-page document published earlier this month, Heinz Heckhausen, the chairman who is also the director of the Max Planck Institute for psychology at Munich, says that the average length of university studies in West Germany is much greater than in comparable countries. His report advocates a basic four-year course with only a few exceptions.

A century ago, most university students studied for only three years, but since then their courses have been lengthening steadily. The Heckhausen report says that the average length of study in social science and economics amounts to 11 half-year semesters. In mathematics, natural science and medicine, students spend an average of 12.8 semesters at university. Chemistry students, who usually finish up with a doctorate, spend no fewer than an average of 19 semesters at their universities, which means that they are usually 31 years old by the time they leave.

Under the new proposals, the content of university courses would be chosen to give students "a high degree of professional mobility" and would be complementary to professional practice. Courses would ordinarily last for four years, but there would be an extra three months of study for certain courses with exceptional needs.

The proposals fit in well with the recent discussions of the proposed new university law *Hochschulrahmengesetz* (see *Nature* **316**, 96; 1985 and **317**, 717; 1985). Significantly, however, they are addressed not to students but to academic boards, to universities and to professors. This may be the first time in the long discussion of this problem that responsibility has been shifted from students to officials.

The underlying difficulty is that the threefold increase of student entrants to West German universities, from 65,000 in 1950–61 to 155,000 in 1984–85, has not been accompanied by reorganization of university courses. As long ago as 1966, the then minister of education in the federal government, Hans Leussink, advocated that action should be taken, but almost the only tangible result was that

discussions of the problem were made more prominent.

The Keckhausen report is critical of proposals that have emerged from the Studienreform Kommission in the past few years, which has suggested ways of reducing the length of 10 university courses (excluding the time occupied by final examinations) to 10 semesters, a further four courses to nine semesters, but with courses in architecture remaining 12 semesters long. According to the new report, these are inappropriate recipes.

The Wissenschaftsrat's own proposals rely crucially on the assumption that a small proportion of the students completing first "four-plus" courses at universities would go on to graduate studies. The Heckhausen report, however, contains no specific proposals as to how the existing university courses would be shortened, and there are many who argue that shifting the problem to the universities is no solution.

Thus Theodor Berchem, the president of the Westdeutsche Rektorenkonferenz (the standing committee of university heads), has warned that too much should not be expected from the Wissenschaftsrat's proposals, which have been realized "only on paper". He points out that students whose employment prospects are not bright or who seek the high grades required for entry to the civil service will be tempted to prolong their studies to improve their grades by changing "the places after the decimal point".

According to WRK, there will be a change only if industrial companies are prepared to relax their demands of leaving students for high qualifications. Others point out that the universities have not yet been able to demonstrate that they can reduce the length or specialization of their courses, while the Gewerkschaft Erziehung und Wissenschaft calls the proposals "hollow" and "cobbled together", saying that the necessary conditions for their implementation must be a sufficiently generous payment to students and better professional prospects.

On the other hand, the federal minister for education, Dorothee Wilms, has praised the recommendations, emphasizing that the central and regional governments are in agreement on the issues raised by the report. She says that the federal government has already offered the regional governments assistance with the preparation of new graduate courses, but that it will be for the universities themselves to carry through the bulk of the reform. She hopes that the growing competition among universities for students will be a powerful incentive for change.

Jürgen Neffe

French elections

Chevènement's last hurrah?

M. JEAN-PIERRE Chevènement, the architect of the French socialists' expansionary science policy, startled French voters on the eve of last Sunday's general election with the proposal that the *grandes écoles* should be doubled in size. Much of the interest of this proposal lies in its source. Chevènement is the leader of Ceres, the most substantial left-wing grouping within the Socialist Party, and as such might just as well have proposed that the *grandes écoles*, the training grounds of the French élite, should be abolished.

Since the beginning of his two-year spell as French minister of research and industry five years ago, Chevènement has been an ideas man. Now that the voters have proved the opinion polls right, and have robbed the socialists of their hold on the executive branch of the government, there will be no chance to tell whether the proposal was seriously intended or merely an election ploy. But this is not the first time that Chevènement has departed from the ideology of the left: as minister of education during the past two years, he has been responsible for a number of innovations — such as the singing of the Marseillaise every morning in the *lycées* — which are more popular with the right than the left.

This may not be as inconsistent as it sounds. Chevènement now calls himself a "republican élitist"; what he did last week was merely to endorse a report he had commissioned from Mme Josianne Serre, director of the École Normale Supérieure de Sèvres, whom he had asked to find ways of doubling the annual entry to the *grandes écoles*, now a meagre one per cent of the annual entry into higher education. What Serre (and Chevènement) now propose is that entry should not depend exclusively on success in the "baccalauréat C", with its emphasis on mathematics and physics, but on the results of a broader school-leaving examination.

The Serre report also advocated that the proposed doubling of the population of the *grandes écoles* should be brought about not only by increasing the size of existing institutions but by creating more of them, concentrating on technical fields other than the branches of conventional engineering to which most *grandes écoles* are at present dedicated. This coincides with the present inclinations of the *grandes écoles* themselves, for which reason there is a chance that these influential institutions could make the changes happen even if the new government declines Chevènement's offer to serve even in a right-wing government under suitable conditions.

Robert Walgate

Correction

In last week's *Nature* (**320**, 101; 1986), the name of John McTague, the acting director of the White House Office of Science and Technology Policy, was misspelt. □

Embryo research

SIR—I cannot claim to have read every word submitted to the Warnock Committee of Inquiry into Human Fertilisation and Embryology, of which I was a member, but I am reasonably sure that at least in our discussions the word “pre-embryo” was never used. Certainly we were all well aware that the human embryo for the first two weeks of its existence, and even more, bears no visual resemblance whatever to the later embryonic and fetal stages, but I can recall no efforts either within or from outside the committee to redefine the early stages as not an embryo. Within the past year, however, the word “pre-embryo” has been creeping in. For instance Maxine Clarke (*Nature* 319, 349; 1986) reports that nine research councils in Europe agree that research should be restricted to the “pre-embryo” and that the British Medical Research Council is supporting research to develop a test to identify healthy pre-embryos.

If research on embryos were an uncontroversial matter, and if scientists were generally of the opinion that the new terminology helped their understanding, nobody would have many qualms at the name change. But those who are introducing “pre-embryo” into the vocabulary know full well that the research is indeed contentious and that fundamental issues have yet to be resolved. They complain, with justification, when embryos are described as “unborn children” in hostile parliamentary bills, but they are themselves manipulating words to polarize an ethical discussion.

The central issue is whether it is justifiable, once an egg has been fertilized, for anyone to perform experiments on the resulting embryo when those experiments are not in the best interests of that particular embryo. This is a complex and profound issue. The introduction of cosmetic words does not help.

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David Davies was Editor of *Nature* from 1973 to 1980.

Price of star wars

SIR—Joseph Hertzlinger (*Nature* 319, 8; 1986) has drawn attention to an aspect that I had overlooked in my letter (*Nature* 317, 470; 1985) about some of the consequences of the US Strategic Defense Initiative (SDI). However, the plutonium scattered into the atmosphere by the non-nuclear explosion of a Soviet missile will not only increase the background alpha-radiation by about 50 per cent; unlike naturally-occurring radon gas, it will be unevenly distributed in the troposphere

and, as plutonium oxide, it will be in solid form. A litre of inhaled air containing 1 µg of the oxide could be lethal; a litre of air free of the plutonium but with the average global proportion of radon gas would have less than one ten-millionth of the radioactivity of 1 µg of plutonium and be virtually harmless.

Probably more important than the scattering of radioactive debris in the atmosphere, however, are the consequences of the arrival at ground level of guidance-damaged missiles and parts of exploded warheads containing plutonium-239. The rumour caused by that part of the recent report of the Australian Royal Commission dealing with the British A-bomb crash tests at Maralinga related to the scattering of a few kilograms of plutonium over a test site in the Australian desert. In the SDI scenario, the amount of plutonium could be thousands of kilograms and the “test site” could be populated Europe.

R.V. HARROWELL

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SIR—R.V. Harrowell (*Nature* 317, 470; 1985) raises an interesting question about a successful US defence system, but his conclusions may be misleading. Consider the more extreme case where 15,000 kg of plutonium-239 is uniformly dispersed throughout the $2 \times 10^6 \text{ km}^2$ of Europe (bounded by 5°W to 15°E longitude and 45°N to 55°N latitude). It is assumed that an operable ATBM (anti-theatre ballistic missile) defence system intercepts 100 per cent of the incoming warheads at very low altitudes. A simplified pathway to human intake will be used in which only the effects on the lungs due to inhalation of insoluble plutonium-239 are considered. The particle size of the plutonium-239 will be assumed to be about 1 µm AMAD (activity median aerodynamic diameter). The deposition velocity is taken to be about 1 cm s^{-1} , and the average breathing rate for humans is about $20 \text{ m}^3 \text{ per day}$. A rough estimate of the average mass of plutonium-239 that might be inhaled per person is

$$\frac{(15,000 \text{ kg}) (20 \text{ m}^3 \text{ d}^{-1})}{(2 \times 10^6 \text{ km}^2) (1 \text{ cm s}^{-1})} = 0.17 \mu\text{g}.$$

The committed dose per unit intake of inhaled plutonium-239 is $16 \mu\text{Gy Bq}^{-1}$ (ref. 1) (which converts to $37 \text{ mGy } \mu\text{g}^{-1}$). Thus, the dose commitment per person within the area of interest would be approximately 6 mGy. Since the ICRP (International Committee on Radiological Protection) limit for radiation workers is 25 mGy (ref. 2), the doses that most people would receive are not lethal. There would certainly be an increase in the long-term effects (for example, lung cancers), but the short-term mortality rate would not be significantly changed.

The question of whether one dies quick-

ly by nuclear annihilation or slowly by plutonium poisoning is not the central issue. The more realistic threats of future anti-ballistic missile (ABM) (and ATBM) defence systems deserve more attention.

The ABM Treaty of 1972 has recently raised a dilemma regarding the US Strategic Defense Initiative because it is only a bilateral measure and does not address the ATBM issues. This substantive deficiency must be addressed in the near future lest the heavens surely become the battleground of the twenty-first century.

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1. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). *Ionizing Radiation: Sources and Biological Effects*. 1982 Report to the General Assembly. Annex E. p. 239.
2. Recommendations of the International Commission on Radiological Protection. *Annals of the ICRP* ICRP Pub. 26, vol. 1, no. 3, January 1977.

Women back to work

SIR—In his article on career breaks (*Nature* 319, 522; 1986), Richard Pearson remarks that “many more women would return if it could be to part-time work”. The academic world has been slow to recognize this important condition, but the situation is changing. For example, the Science and Engineering Research Council has given positive support for part-time appointments to its fellowships and grant-funded positions.

I have initiated a scheme of part-time fellowships for women wishing to return to academic research in science and engineering. The first group of fellowships has been funded by British Gas, British Telecom, GEC, the Leverhulme Trust, the Institute of Physics, ICI and BICC. Donations have been received from Rank Xerox and British Aerospace.

Over a quarter of a million pounds has so far been raised and the first seven fellows have been appointed. Ninety applications have been received; well over half of the applicants have PhDs and well over a third have industrial experience. Two essential features of the scheme are that the fellowships are part-time and retraining is available. The university departments involved have been most helpful.

It is hoped that this scheme will serve as a pilot project for the return of qualified women to industry. It is clear that returners should be offered flexible working patterns and that contact must be made through local press and radio.

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Heavy-ion positrons explained

The surprising observation of mono-energetic positrons in heavy-ion collisions may not be the signature of a novel particle but merely a sign that classical physics has been overlooked.

THAT apparently small phenomena perceptively observed can lead to huge conceptual changes is a large part of the excitement of science. Newton's apocryphal falling apple is one example. Another is the recognition, by Meitner and Frisch (*Nature* **143**, 239; 1939) that the presence of barium in the products of the reaction of neutrons with uranium must mean something odd, such as the fission of heavy nuclei.

So it is inevitable that there should have been suppressed excitement for well over a year about an unexpected phenomenon associated with the collision of heavy ions which appears first to have been recognized at the heavy-ion accelerator at Darmstadt, the West German town just south of Frankfurt which, since last weekend, will briefly be best known as the site from which the European Space Agency coordinated the flight of the Giotto spacecraft to Halley's comet.

The circumstances are these. The Darmstadt laboratory, in German the Gesellschaft für Schwerionenforschung, has a longstanding (and successful) interest in the creation of artificial heavy nuclei, to which end it is equipped to collide heavy ions with each other so energetically that the Coulomb repulsion of their positive charges are overcome. The surprise, first reported in 1983, is that if the combined charge of the colliding ions is great enough (more than 163 units) the collisions are accompanied by the production of positrons with a precise energy (measured in the rest frame of the colliding ions).

Specifically, the energy distribution of the positrons appears to have a peak at an energy of 336 keV, which is itself apparently independent of the nature of the colliding ions. The width of the distribution is 75 keV or so, implying that the positrons are produced from a source whose lifetime is long by the yardstick of the time taken by the nuclear collision of the ions, estimated at 10^{-21} seconds or so.

The excitement generated by these observations is easily imagined. The temptation has been irresistible to speculate about the properties of the unknown particles that are supposedly formed in the collision of heavy nuclei and which, by their radioactive decay, yield the positrons that are observed. One of the favourite candidates has been the axion, the particle that should exist if parity and isotopic spin are not conserved in weak

nuclear reactions (see *Nature* **319**, 717; 1986). There has even been the beginnings of a controversy on the subject, with some people saying that axions are produced because of their coupling to electrons, while others have held that the production process involves the interaction of hadronic particles, nuclear matter proper.

It now seems more probable that both camps are wrong, and that the unexpected observation of positrons in heavy-ion collisions is not so much evidence of a novel state of matter but rather a sign that even the people who should know much better have overlooked what should now be elementary physics. That, at least, is the implication of a modest article by Cheuk-Yin Wong, from the Oak Ridge National Laboratory in the United States, in the 10 March issue of *Physical Review Letters* (**56**, 1047; 1986).

Wong's argument is essentially quite simple. It turns on what should be by now Dirac's familiar description of what the vacuum is like. The point is merely that when Dirac first recognized more than half a century ago that a properly relativistic equation for the quantum motion of a free electron would admit of energies that are negative as well as positive, he was compelled to save the appearances by supposing that all the negative energy levels were in the real world completely occupied. If this were not the case, ordinary free electrons would forever be making the transition from a positive to a negative energy level, with the result that we would live in a world filled with radiation more energetic than the equivalent of 500 keV, which is half the smallest gap between a positive and negative electron energy level.

As the world knows, Dirac's view, strange though it may have seemed at the time, has been a great success. A positron is nothing but a hole in the sea of ordinary electrons with negative energy. The annihilation of positrons by ordinary electrons is the occupation of such a hole by an ordinary electron. The creation of an electron pair by a sufficiently energetic photon (whose energy must exceed the equivalent of twice 500 keV) consists simply of the promotion of an electron from the negative energy sea, leaving a hole that seems to be a positron, into an ordinary state of positive energy. Particle field theory is full of many more sophisticated applications of the same principle, under the banner of the "polarization of the vacuum" by suffi-

ciently strong fields of force. (Near a black hole, gravitational forces produce other particles than electrons in this way.)

In essence, what Wong now says is that the positrons observed in heavy-ion collisions arise in much the same way. Dirac's concept of a sea of electrons is valid only in the absence of electrical or magnetic forces, and must therefore be modified in the neighbourhood of a nucleus. Wong points out that the energy of the two electrons in the lowest bound state in an atom whose atomic number is greater than 150 is negative, which is another way of saying (a little crudely) that its binding energy to the nucleus of the atom is greater than its rest energy, that due to its mass.

Indeed, Wong argues that for a sufficiently heavy ion entirely devoid of electrons, it would be energetically favourable that there should be created two electron-positron pairs, each of them crammed into the ground state of the nucleus (which would not be forbidden by Pauli's exclusion principle).

The extension of the argument to heavy-ion collisions is straightforward. Electron-positron pairs created in this way in the ground states of colliding ions would be shaken loose during the collision and thus left to their own devices. In the centre of mass of the system, they would be virtually at rest. Only mutual annihilation would be feasible. So why should not the electrons and positrons annihilate each other in pairs? Sometimes, no doubt, they do, producing 500-keV radiation that will promptly be lost in the background. The more noticeable process, according to Wong, is that a single pair should annihilate each other, producing a single photon and transferring the excess momentum to a second positron that happens to be in the neighbourhood.

The surprise in this simple calculation is that the numbers turn out to be just right. The calculated kinetic energy of the surviving positron is 341 keV. Wong predicts that the accompanying photons (which might be detected by a coincidence experiment) should have an energy equivalent to 681 keV. There should also, he says, be observed the products of the inverse process in which excess momentum is transferred to an ordinary electron. That at least is something for which the experimentalists can hope to look, while they berate themselves (not to mention their theorist advisers) for having forgotten about Dirac.

John Maddox

Developmental neurobiology

Axon outgrowth in vertebrates

from P.H. Taghert and J.W. Lichtman

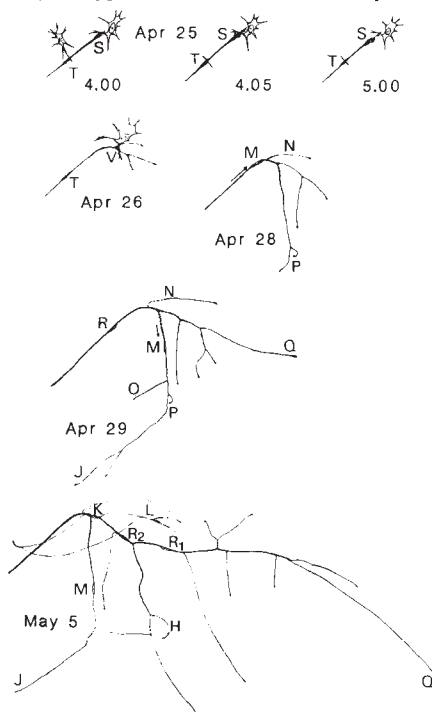
CARL Speidel observed the growth of single nerve fibres in the tails of living tadpoles more than fifty years ago¹. Despite the ease with which he could watch axons over long time-periods (see figure) this line of enquiry lay more or less dormant until very recently. Perhaps one reason that *in situ* studies of axon outgrowth have been avoided is that it was not clear what such observations should explain — the relation between axon outgrowth and the specificity of neuronal connections was not always as evident as it now seems. Indeed, Paul Weiss promulgated for many years hypotheses arguing that axons either grow everywhere (resonance in the patterns of the activity of pre- and post-synaptic cells generated the specificity)², or grow anywhere (the specificity occurring as a secondary step when central connections became specified by the targets axons contacted)³. Although it is now clear that at least some axons transiently project to multiple regions (see ref. 4 for review), recent work, including two reports in this issue^{5,6}, shows that at least some axons are guided to their intended targets.

It is now generally agreed that the cellular and molecular mechanisms underlying the specificity of neuronal connections concern two general phenomena. The first is the guidance of developing axons to the neighbourhoods of the appropriate targets; the second is the formation and subsequent refinement of synaptic connections. The importance of axon guidance for synaptogenesis is obvious: neurones are capable of making synapses only with those targets that their growing axons can contact. It follows that to elucidate the basis of specific synaptic connections the mechanisms that guide developing axons through the embryonic cellular environment must be understood.

The articles by Harris⁵ and by Eisen *et al.*⁶ in this issue help to elucidate these mechanisms in vertebrate embryos at a time when we are becoming increasingly knowledgeable about axon outgrowth in invertebrates. The application of cellular techniques to the study of developing axons and growth cones *in situ* in insect embryos⁷⁻¹⁰ generally support the hypothesis that the growth cones of individual neurones (via filopodia) actively select among many available cell substrates specific pathways on which to extend. Identified neurones make stereotyped choices of substrates at specific locations in the developing neuropil or periphery. These observations of normal growth-cone behaviour in developing embryos have

generated the view that the most critical environmental factor is the set of cell-surface 'markers' whose existence is inferred from the stereotyped and highly selective pathway choices made by individual growth cones⁹ and the equally selective filopodial contacts at the precise times that pathway changes occur¹⁰.

These inferences (formalized as the labelled pathways hypothesis⁹) are in many ways analogous to Sperry's chemo-affinity hypothesis that sought to explain the precise connection between cells of the retina and the tectum in vertebrates. An experimental test of the labelled pathways hypothesis shows that specific



Extension of a growth cone and its branches over several weeks in a regenerating tadpole tail (from the original work of Speidel¹).

ablation of particular neurones and mesodermal cells causes the growth cones of the neurones that normally choose the ablated cells as substrates either to stall or to select a different pathway. It is thought that the major factor controlling this directional movement of growth cones consists of specific interactions between a growth cone and its neighbouring substrates. In this view, pathway alterations result from sampling the cell surfaces of available substrates and substrate preference reflects the greatest adhesion between two cells whose membrane labels are most 'compatible'.

The study of axon guidance in insect embryos has been so fruitful because of

the accessibility and identifiability of embryonic neurones during the stages of development at which the critical events occur. It has been much more difficult to study vertebrate embryos. Recently, Lynn Landmesser and her colleagues have described the behaviour of chick motor neurones as they navigate out of the spinal cord towards their muscle targets both during normal development and following experimental manipulation^{11,12}. Their interpretation of the events suggests that the vertebrate axons find their specific targets by growing along stereotyped pathways.

Eisen *et al.*⁶ return to the approach initiated by Speidel by examining the growth of individual motor axons in the spinal cord of living zebra fish embryos. They have used the optical clarity of this embryo and the intracellular injection of fluorescent probes to describe the complete sequence of events as the growth cones of the motor neurones first navigate out of the spinal cord. Consistent with the behaviour of growth cones in insect embryos and the interpreted behaviour of chick spinal motor neurones, these cells appear to make cell-specific pathway choices. Similarly, within the developing fish spinal cord, the first interneurons to project axons do so along stereotyped substrates¹³. The stereotyped, precise and cell-specific nature of the axonal guidance suggests direct parallels with similar phenomena in insect embryos.

Harris⁵ also examined the development of stereotyped axon pathways in the vertebrate nervous system. Once again, the novelty of the study arises from analysis performed not just after growth cones have finished navigating, but also during the formation of these pathways. Retinal neurones placed at ectopic locations in the developing brain of the South African clawed toad (*Xenopus*) appear to home-in to their normal target region in the developing tectum. Especially noteworthy is the evaluation of the trajectory of ectopic axons during their corrective action. Harris demonstrates that the axons do not fail to navigate correctly even at times soon after axonogenesis, and it is only when the primordium of the retina is placed at long ectopic distances from its prospective target that evidence of 'error' is found.

The ability of developing vertebrate neurones to correct experimental misplacement up to a certain spatial point as demonstrated by Harris is reminiscent of events in the chick spinal cord¹¹. Interestingly, the interpretations differ slightly in their emphasis: Lance-Jones and Landmesser¹¹ suggest that motor neurones navigate under normal circumstances by deciphering some aspect(s) of the local environment at what are now called decision points¹². Past a certain distance, access to the proper local environment is no longer possible and thus mistakes in axon projection occur with increasing regular-

ity. Harris⁵ emphasizes the fact that retinal neurones seem to show directional predilections almost anywhere when they are ectopically placed in the developing brain. Hence, he predicts that the positioning of cell-surface signals (or strength of a diffusible gradient) necessary for the guidance of particular axons are not restricted to decision points but are instead distributed to many neuronal locations.

Perhaps the two sets of interpretations could be reconciled by considering the absolute distances of the ectopically placed neurones from their normal targets. Are the dimensions of the developing *Xenopus* brain very much greater than those of the local environment deciphered by chick motor neurones?

In the examples we have described here (and in others; see, for example, ref. 13), the picture is beginning to emerge that the earliest neurones to project axons in the developing nervous system of vertebrates do so in a stereotyped manner. The mechanisms that underlie this form of

development, and their relationship to potentially analogous mechanisms of invertebrate systems, are fast becoming subjects of more than simple speculation. □

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Archaeology

Beginnings of English village life

from Richard Hodges

TWENTY-FIVE years ago archaeologists generally believed that the first English villagers of the fifth to seventh century AD lived in 'pit-houses' in a primeval condition. Improved excavation techniques in the 1960s, however, brought a new image of the early English to light. By excavating large areas rather than trenches it became apparent that the remains of post-built halls existed alongside these sunken pit-houses. Moreover, the new excavation techniques revealed a material culture pertaining to daily life as opposed to the common picture of a barbaric society. Although contemporary cemeteries contained bodies inhumed with swords, shields and spears, the first excavated villages by contrast seem to have been places of rich tranquility. Such points have been revealed by one of the first of these village excavations, at West Stow, Suffolk¹.

This village, one of several discovered from this era, was situated in the Lark Valley, north of Bury St Edmunds. Excavations and survey show that the Roman villas in the area were falling into decay by about AD 400, well before the imperial government left Britain; it is equally clear that West Stow, like several other communities, was founded in the wake of this traumatic decision². The new report charts the sequence of the community from the early fifth and seventh centuries, when it was probably abandoned in favour of a nearby site. At any one time there were about three halls accompanied by up to about a dozen small

sunken huts. Each hall was constructed of posts set deeply into the ground with walls clad presumably with wattle and daub, whereas the sunken huts were smaller buildings, probably with lean-to roofs over a sunken floor. It is now thought that

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Reconstruction of the West Stow village (courtesy of Suffolk County Council).

the halls were for accommodation, while the sunken huts were used for domestic crafts and storage. The halls, however, are unlike contemporary German long-houses and much closer in style to the peasant farms of Roman Britain³.

The absence, until the seventh century, of ditched enclosures around each farm unit is also strikingly at variance with continental village patterns in this period^{4,5}. Yet the material culture is a classic early Anglo-Saxon assemblage. The stamp-decorated sixth century pottery, the bone combs and the jewellery are consistent, if poorer in quality, with the assemblage found in the cemetery at West Stow as well as other Anglian cemeteries. Faunal and botanical assemblages also show a pattern

of mixed farming with pigs making up a fairly high proportion of the livestock (about 20 per cent), while it appears that spelt, typical of Roman farms, had been replaced by wheats and rye (pages 86, 103 of ref. 1; and ref. 6). The subsistence strategy suggests a localized regime of low intensity compared with the more specialized, productive agrarian policies of Roman times. West Stow must have had a population numbering about 20–30 until the early seventh century when the village was abandoned. Note that ditches enclose the final-phase buildings, as if in the era of St Augustine and with the coming of Christianity, attitudes to property altered.

The survey of the Lark Valley indicates that West Stow was typical; similar villages existed at regular intervals following the desertion of the Romans. The material culture suggests that their occupants were English, whereas the buildings hint that they were descendants of Romano-British peasants adapting to the changes.

For almost two hundred years a largely domestic mode of production was sustained with involvement in regional exchange being only on a modest scale. At the same time, some elements of warrior status occur in the cemetery in the form of spears and shields despite the egalitarian agrarian character of the village buildings. Some time before AD 700 almost all the early Anglo-Saxon sites in the valley were deserted in favour of new loci, most of which are still occupied today (page 170 of ref. 1). These divisions look purely functional and economic, with river frontages, water meadows and arable land, backing onto high drier sheepwalks. However, other recent village excavations such as those at Raunds (Northamptonshire) and Wharram Percy (North Yorkshire) show that the parish church and its bounds were features of the tenth century and the socio-economic changes of that era^{7,8}.

The excavations at West Stow provide an image of the transitory age between the collapse of Roman Britain and the formation of the Christian kingdoms of the seventh century. It is too soon to challenge the traditional history, yet, just as these excavations reveal that Anglo-Saxon farmers did not inhabit pit-houses, it is clear that their agrarian way of life constitutes a significant chapter in the evolution of English medieval life. □

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Prochlorophytes

Origins of chloroplasts

from A.E. Walsby

OUR Victorian forebears did such a good job of describing the major groups of plants, animals and microbes that it is now a particular delight to find even a new species, let alone a representative of a new family or higher order. A decade ago, R.A. Lewin's discovery of the Prochlorophyta^{1,2}, a new division of photosynthetic microbes, was therefore quite momentous, particularly when it was realized that this might be the missing link in the evolution of chloroplasts. The study of prochlorophytes since then has been hampered because only symbiotic forms have been found, none of which can be grown in culture³. But on page 262 of this issue³, T. Burger-Wiersma and her colleagues report the discovery of the first free-living prochlorophyte from the Loosdrecht lakes of the Netherlands.

The *Prochloron* found by Lewin is an oxygen-evolving photosynthetic prokaryote that possesses both chlorophyll *a* and *b*, the photosynthetic pigments of green plants⁴. The serial endosymbiosis theory⁵ claims that eukaryotes — all those organisms whose cells have a true nucleus and membrane-bound organelles — arose by the symbiotic merger of prokaryotic organisms, and that in eukaryotic plants the chloroplast developed from a cyanobacterium (blue-green alga). This particular aspect of the theory had one drawback: although cyanobacteria also perform a similar type of photosynthesis based on chlorophyll *a*, they lack chlorophyll *b*, the pigment that is involved in the second oxygenic photosystem of plants (photosystem II). They have instead a photosystem based on pigmented phycobiliproteins which, although functionally homologous, must have evolved by a different route. It is probable that cyanobacteria were the precursors of chloroplasts in red algae, which have almost identical phycobiliproteins, but what of chloroplasts in all the other plant groups? Did they develop from primitive red algae, evolving chlorophyll *b* to replace the phycobiliprotein pigments of photosystem II, or were they products of a separate endosymbiosis involving prochlorophytes⁶ that had already adopted such a replacement? One might even ask which came the first, the cyanobacteria or the prochlorophytes?

In an attempt to answer such questions Lewin's *Prochloron* has been investigated from every conceivable angle. The first *Prochloron* was found on the surface of didemnid ascidians (sea squirts, sedentary hemichordates that produce sponge-like incrustations on rocks) in the intertidal region of Baja, California. Other pro-

chlorophytes that differed in size and other minor respects were soon discovered in tropical waters but they, too, were always present in association with these same animals. The failure to grow prochlorophytes in culture even produced some anxiety that they might not be complete organisms but chloroplasts, escaped from some unidentified algae and enslaved by their sea-squirt host. In the absence of cultures there was no option but to analyse the few prochlorophyte cells that could be obtained from the sea-squirts.

Structural and biochemical analyses of precious milligrams of material show *Prochloron* to be closely related to cyanobacteria⁷. The organism possesses a peptidoglycan wall, diagnostic of eubacteria, and polyhedral carboxysomes (stores of the CO₂-fixing enzyme) peculiar to autotrophic prokaryotes. The carotenoids and lipids are similar to those in cyanobacteria, as are other features of the cytoplasm. The most telling comparison is that of their ribosomal RNA sequences, which indicate that *Prochloron* is phylogenetically closer to cyanobacteria than to the chloroplasts of green plants⁸. One important finding⁹ was that the symbiotic *Prochloron* had a DNA genome size of relative molecular mass 3.6×10^9 , similar to that of many cyanobacteria and 30 times greater than the residual genome of chloroplasts, which is insufficient to support an independent existence by these organelles.

In one respect the prochlorophytes did resemble green chloroplasts more closely than cyanobacteria: their thylakoids (photosynthetic membranes) were paired or stacked. However, this may be a quasi-

mechanical consequence of the presence of chlorophyll *b* and the absence of phycobilisome structures, which in cyanobacteria prevent the close juxtaposition of neighbouring thylakoids. It may not, therefore, be of any phylogenetic significance.

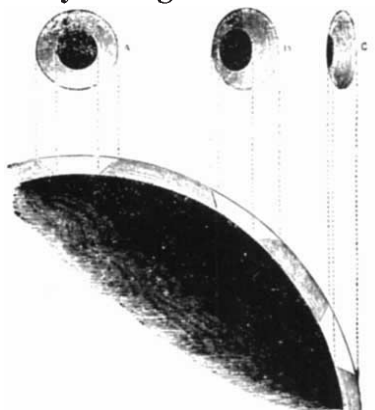
With the newly discovered free-living prochlorophytes, which morphologically resemble filamentous cyanobacteria, it will be possible to make even more fundamental comparisons. For example, although Burger-Wiersma *et al.* find no trace of phycobiliproteins in the cells, it would still be interesting to see if there are DNA base sequences homologous with the structural genes for these proteins that have been isolated from several cyanobacteria during the past year. Even more exciting is the prospect of using recombinant DNA technology to transfer the genes required for a chlorophyll *b* photosystem II into phycobilisome-disabled mutants of cyanobacteria (and vice versa). These experiments would define the amount of information required to evolve such a photosystem and in this way shed light on the origins and affinities of these photosynthetic prokaryotes.

In just one respect the discovery of the free-living prochlorophyte has done a disservice to biologists: previously, *Prochloron* could be obtained only by visiting a sun-drenched beach in Baja or a tropical island. A surprising number of biologists not noted for their field work found such visits worthwhile. Now they can investigate the biochemistry of a prochlorophyte in the comfort of the laboratory. □

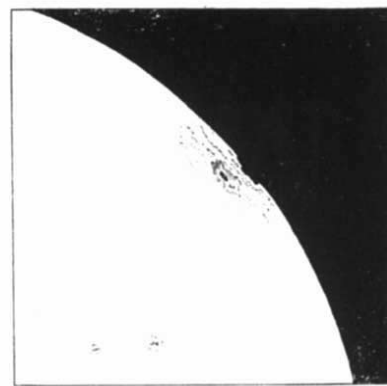
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100 years ago

from *Nature* **33**, 469, 18 March 1886

Appearances presented by spots. A is the centre of the disk; B between centre and limb; C, near the limb.



Copy of part of a photograph taken at Dehra Dun in 1884, showing a sunspot passing over the Sun's edge

In the large photographs now secured at Meudon and in India, showing the sunspots, I am glad to say almost every day now, on a scale of 8 inches to the sun's diameter, we get wonderful records of what a spot really is, and how it changes.

Gene therapy

Desperate appliances

from Miranda Robertson

It was widely prophesied last year that the first clinical trials of gene therapy would be under way before the end of 1985 if obstructive action by the US Food and Drug Administration (FDA) did not prevent them¹. As it turns out, however, gene therapists have been thwarted not by the FDA but by the biology of the retroviruses on which they depend for gene transfer. That the paper published by Hock and Miller on p. 275 of this issue² constitutes a substantial advance in the development of retroviral vectors for human gene therapy is a measure of how far away it still is.

The paper reports the transfer of a drug-resistance gene to human bone marrow cells. What makes it important is that this is the first time that has been achieved in the absence of infectious helper virus (which could contaminate the bone marrow cells and preclude their use for gene therapy); and that the drug-resistance gene is expressed in the transfected cells. The ingenuity that has gone into the design and construction of the retroviral vectors that have made this possible is awe-inspiring; but it seems it is not enough. What are the problems of the technology, and what now are the prospects for putting it to use?

Prospects

No-one expects in the foreseeable future to be interfering with human fetuses: the immediate hopes for gene therapy are concentrated on those diseases that could in principle be treated by the manipulation of haemopoietic cells of the bone marrow, which are relatively easily removed and replaced and provide a self-renewing population of cells for the propagation of transferred genes. (A comprehensive account of the prospects for gene therapy is given in ref. 3.) The diseases that are susceptible to this approach include disorders of the haemopoietic cells themselves — hereditary anaemias and immune deficiencies — and single-protein deficiencies such as haemophilia in which the release of the missing protein into the bloodstream would correct the defect.

Its success depends on the efficiency with which a normal copy of the defective gene can be introduced into bone marrow cells in such a way that it will be expressed as protein in their progeny. The efficiency of the vector is crucial because if the gene is to persist in the bone marrow after it has been returned to the patient it must enter the self-renewing stem cells that are responsible for replenishing the entire haemopoietic population. These stem cells have been estimated to com-

prise 0.01 per cent of bone marrow cells, and there is no guarantee that all of those transfected will express the gene; so to be certain of getting useful amounts of the normal gene product it may be necessary for the vector to enter virtually 100 per cent of the cells. Only retroviral vectors are capable of this order of efficiency; but an elaborate series of manoeuvres has been necessary to devise a vector that will deliver the gene without leaving the cells full of infectious virus. Briefly, the trick is as follows.

The vector contains only the gene to be transferred; the sequences necessary to insert itself into the DNA of the cell and ensure transcription of the gene it carries (both contained in the long terminal repeated sequences (LTRs) at its ends); and a packaging signal — an uncharacterized sequence that enables the viral genome to be packaged into its protein coat and escape the cell as an infectious particle. Because it does not contain the coat-protein genes, however, it is incapable on its own of generating infectious particles. Large quantities of infectious virus must nonetheless be generated for the efficient transfection of bone marrow cells, and this is made possible by introducing plasmids containing the vector sequences into cell lines that harbour what are known as helper viruses. The helper viruses still have their coat- and envelope-protein genes, so that when the vector genes are replicated in the cell, they can be packaged in the helper coat proteins. The packaging cell line is incubated with the bone marrow cells, which the vector particles are able to enter. Once in the bone marrow cells, the vector genome becomes integrated in the cellular DNA where, because it has no coat-protein genes of its own, it cannot give rise to further infectious particles. With an intact helper virus, helper particles are produced along with the vector particles; this is not acceptable for human gene therapy and can now be avoided by the use of helper viruses from which the packaging signal has been deleted so that they cannot package their own genomes and are trapped in the cell lines they are grown in.

Such a combination of packaging-defective helper and retroviral vector has been successfully used to introduce drug-resistance genes into mice, through transfection of the bone marrow^{4,5}; but until now no-one had been able to get it to work in human bone marrow cells, and people were beginning to wonder if the systems that work for mouse cells are for some reason simply unsuitable for use in man.

Hock and Miller have now shown that this is not the case. They used a cell line, PA-12, which contains a packaging-defective helper with a wide host range, to produce a vector carrying the dihydrofolate reductase (*dhfr*) gene, which confers resistance to methotrexate, and have been able to grow drug-resistant cells from transfected human bone marrow.

Problems

The drug-resistant cells that they grew in this way were, as it happens, derived from CFU-GM cells, the progenitor cells for the myeloid lineage. How likely is it that if the system will work for CFU-GM it will also work for the self-renewing stem cell — a rarer and more elusive species? There is no completely satisfactory answer to this question, largely because there is no unequivocal assay for the self-renewing haemopoietic stem cell short of putting it back in the animal, which is not an option where the appropriate animal is man. But experiments with essentially similar systems in mice suggest that if it will work for a late progenitor like CFU-GM then it will work for earlier ones too. It would help to show that drug resistance can be conferred in the same way to other human precursor cells that can be assayed *in vitro* — namely, the erythroid precursor BFU-E and the earlier progenitor cell CFU-MIX, which is the precursor of both CFU-GM and BFU-E. Hock and Miller have almost but not quite done this.

Using two other vectors, they have succeeded in growing drug-resistant colonies of all three progenitor cell types, but only from cultures containing helper viruses. The helper virus in the cultures was an unpleasant surprise: it materialized through recombination between the packaging-defective mutant and the vector during co-incubation, because of a short region of homology between the vector and helper viral sequences (the vector that came out helper-free did not have this region of homology). This rather strikingly illustrates some of the hazards of working with retroviral vectors — people do worry about the possibility of recombination between vectors and endogenous viral sequences in human cells; but although the presence of the helper virus would be an absolute bar to the therapeutic use of the cells, it does not account for the essential observation that drug resistance can be transferred to three types of haemopoietic progenitor cells: expression of the transferred genes can be detected in the bone marrow immediately after transfection, when it can only be due to the activity of the vector.

Nor does the presence of helper virus seem materially to affect the efficiency of transfection, which Hock and Miller estimate at about 3–10 per cent for CFU-GM. This is almost certainly too low for practical purposes, partly because it is clear

from experiments with mice that only a small proportion of those cells containing the gene are likely to express it. Indeed, expression in general has been a major problem, especially in human bone marrow cells, which is another reason for the importance of the results reported by Hock and Miller. On the other hand, again, the fact that the drug-resistance genes are expressed even in the CFU-MIX does not necessarily mean they would be expressed in self-renewing haemopoietic stem cells. This is not a quibble: it is known that the viral sequences can induce permanent suppression of viral gene expression in embryonic cells, and although the mechanism is unknown it is suspected that the viral LTRs may be responsible. This raises the unwelcome possibility that the failures of stable gene expression in bone marrow are due to analogous mechanisms that cause stem cells to suppress everything under the control of the viral LTR. Re-engineering the LTR so as to retain its essential functions in vector replication and integration, but eliminate the suppressive effect, would not be trivial.

It is probably fair to say that problems of efficacy are for the moment taking precedence over questions of safety. If you cannot make a vector work, after all, you will not be tempted to endanger anyone with it. But it is acknowledged that only for the mortally ill would a treatment involving the random insertion of viral DNA in the chromosomes of proliferating bone marrow cells seem justified.

In an ideal world, the introduced gene would be targeted to replace precisely the defective one by site-specific recombination. This would not only eliminate the danger inherent in random insertion: it would also guarantee correctly regulated gene expression — a truly major advance. At present, the hereditary anaemias, which are the only really common genetic

disorders of the haemopoietic cells, are out of reach because precise regulation of globin gene expression is essential and no-one yet knows how globin gene regulation works, still less how to manipulate it.

There have recently been two reports of site-specific recombination of genes introduced into mammalian cells in plasmid vectors^{7,8}, one of them involving a globin gene⁷. But in neither case is the recombination reliable or accurate enough to have any immediate implications for gene therapy (the implications of the globin gene experiment, and its limitations, have been very clearly laid out in these columns by Tom Maniatis⁹). Moreover its efficiency is vanishingly low: it is the specialized and indiscriminate nature of the viral LTR insertional mechanism that makes the retroviral vectors so efficient.

For the time being, therefore, the prospect of gene therapy is limited to those life-threatening genetic diseases that are due to a single gene defect whose effects can be corrected by the insertion of the normal gene into the bone marrow without the need for precise regulation of gene expression; and for which there is no other cure. The consensus is that clinical trials are very unlikely before next year. I can summarize more elegantly with the lines from which I borrowed the title of this article:

"Diseases desperate grown
By desperate appliances are relieved,
Or not at all."
(Shakespeare wrote them.)

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Miranda Robertson is Biology Editor of *Nature*

tends to dull the cutting edge of our analytical tools. Whereas numerical and analytical methods should be kept as simple as possible for the job in hand, some empirical data, which appear opaque to simple techniques, can yield invaluable insights when analysed more thoroughly.

Dahl-Jensen and Johnsen now demonstrate that the measured temperature profile at the Dye-3 bore-hole in the southern dome of the Greenland ice sheet is significantly different from the profile calculated to be in equilibrium with modern conditions. Using a simple heat-transfer equation, the authors show that surface temperatures must have been colder in the past. Furthermore, by assuming constant surface temperature and accumulation through the last glacial cycle, they calculate the combination of the two which would be compatible with the measured profile. Assuming that surface temperatures lay in the range -30 to -35°C , the authors calculate an accumulation value of about 50 per cent of the modern values. The result is probably only the first step in deducing the significance of the temperature profile anomaly. It should be pursued further, as it represents a valuable piece in an important jigsaw puzzle.

Much work has been done on the reconstruction of environments in the North Atlantic region around South Greenland during the last glacial/interglacial cycle. The polewards heat flux in atmosphere and ocean are greater in this zone than any other comparable latitudinal zone of the Northern Hemisphere and the amplitude of change from glacial to interglacial has been greater here than elsewhere. Terrestrial and oceanic evidence has demonstrated strong environmental changes even within the last glacial episode, beginning at about 120 kiloyears ago (BP) and ending about 10 kiloyears BP.

The North Atlantic appears to have remained an 'interglacial' ocean with a circulation similar to that of the present until about 75 kiloyears BP, from when, until about 13 kiloyears BP, glacial circulation dominated, with an outward surface flow from the polar ocean (Ruddiman, B. F. *Bull. geol. Soc. Am.* **88**, 1813; 1977). On land, a major arctic glacial episode appears to have coincided with the early period of interglacial circulation (Boulton, G. S. *et al.* *Nature* **296**, 437; 1982), possibly associated with high accumulation rates on the North Greenland ice sheet (Andrews, J. T. *et al.* *Geology* **2**, 355; 1974), suggesting particularly high moisture fluxes from a still-warm ocean surface into a cooling Arctic. The period between 75 and 13 kiloyears BP was associated with strong mid-latitude glacier growth and arctic glacial decay, reflecting possible drying and cooling of the Arctic as the polar front moved far south, producing deflection of major storm tracks towards the growing mid-latitude ice

Palaeoenvironments

Temperature of the ice sheets

from G. S. Boulton

CHANGES in the surface environment of the Earth on a timescale of 10^3 – 10^6 years are determined by interactions between oceans, ice sheets and atmosphere in response to changes in incident solar radiation. There are two ways of reconstructing these changes: first, the classical geological approach, in which dated physical remains are used to infer past environments by empirical studies of modern controls on their character and distribution; and second, by the demonstration of the disequilibrium between the current state of a system and the factors which should control that state. A splendid illustration

of this 'memory effect' is described elsewhere in this issue (Dahl-Jensen, D. & Johnsen, S. J. *Nature* **320**, 250; 1986).

The memory effect allows the reconstruction of past changes on a timescale of the order of the response time of the system. But the extent to which the memory can be recalled is limited by our theoretical comprehension of the behaviour of the system. The components of the Earth's hydrosphere are not simple systems which react in a simple way to single external causes, but are complex, interactive and intrinsically difficult to analyse explicitly without the excessive empiricism that

sheets and starving arctic ice masses. South Greenland stands in the critical pivotal zone in this north/south see-saw. It would be surprising if the assumption of a stable glacial climate in Dahl-Jensen and Johnsen's model survived the relaxation of restrictions such as constant thickness and flow pattern.

There are probably many natural systems that are analysed in a highly constrained way which would appear very different if some of the constraints were relaxed. There is often much empirical data which can be used to define a boundary condition that can be satisfied by a properly posed, highly coupled, numerical solution in which the interactions are not artificially restricted. In the case of an

ice sheet such as that of Greenland, time-dependent thermomechanically coupled dynamic models, such as those currently being developed, should be applied to ice-sheet evolution to calculate the mass balance and flow changes that would satisfy boundary conditions such as temperature profiles, isotope composition, gas pressure, crustal rebound and glacial geology. But even such a solution would have to be considered for compatibility within a larger system of oceanic and atmospheric changes □

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Cell motility

Regulation by phosphorylation

from J.M. Scholey

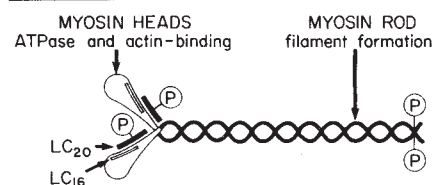
MANY motile activities of cells are believed to be driven by the actin-activated ATPase activity of myosin. In the decade since it was reported¹ that the phosphorylation of platelet myosin increases its actin-activated ATPase activity, similar results have been obtained with many different vertebrate non-muscle myosins. This suggests that changes in the degree of phosphorylation of myosin light chains represent a widespread regulatory mechanism that serves to switch on and off the actin-activated ATPase activity of myosin within vertebrate non-muscle cells. But this simple interpretation of the effects of phosphorylation has now been questioned by P.D. Wagner and J.N. George², using calf thymus myosin.

Thymus myosin resembles other vertebrate non-muscle myosins in possessing two regulatory light chains (relative molecular mass, $M_r \sim 20,000$), one on each head of the molecule, which can be phosphorylated, resulting in a stimulation of actin-activated ATPase activity (see figure)^{3,4}. The actin-activated ATPase activity of thymus myosin varies as a function of the concentration of actin filaments (F-actin) according to Michaelis-Menten kinetics. Wagner and George have now measured the maximum rate of actin-activated ATPase activity (V_{max}) and the actin concentration required to produce $\frac{1}{2} V_{max}$ (called K_{app}), for both phosphorylated and unphosphorylated thymus myosin².

Surprisingly, they find that light-chain phosphorylation causes a 15- to 20-fold decrease in K_{app} , but does not affect the V_{max} , so that the unphosphorylated light chain can be described as a competitive inhibitor of the actin-activated ATPase. At low concentrations of actin, phosphorylation relieves the inhibition, thereby

stimulating the actomyosin ATPase activity. However, as the actin concentration increases, the capacity of this actin to 'compete' out and overcome the inhibitory effect of the unphosphorylated light chain also increases until, at sufficiently high actin concentrations ($>6 \text{ mg ml}^{-1}$), both phosphorylated and nonphosphorylated myosin hydrolyse ATP at similar rates (close to V_{max}).

Light-chain phosphorylation therefore seems to be a feasible mechanism for switching on and off the actin-activated



A vertebrate non-muscle myosin molecule, consisting of two heavy chains ($M_r 200,000$) and two pairs of light chains (M_r s 20,000 and 16,000). The molecule is asymmetrical, comprising two pear-shaped heads attached to a fibrous rod. The heads contain the actin-binding and ATPase sites. Each one contains one of each type of light chain. The function of the $M_r 16,000$ light chains (LC_{16}) is unclear. The $M_r 20,000$ light chain (LC_{20}) has been implicated in the regulation of actin-myosin interaction. P, phosphorylation sites on LC_{20} and on the heavy chains.

ATPase within thymus cells, but only at low cellular F-actin concentrations. About $1-10 \text{ mg ml}^{-1}$ actin is thought to be present in non-muscle cells *in vivo*, but the effective concentration of actin filaments in localized regions of the cell is unknown.

Light-chain phosphorylation also stimulates the polymerization of thymus myosin into filaments *in vitro*, but it is not known whether the effect is physiologically relevant⁵. Whether thymus myosin,

like several other vertebrate non-muscle myosins, can also be phosphorylated on its heavy chains (see ref. 6) is not yet known, but if so, this modification would be another possible mechanism for regulating its activity⁷. For example, the stimulation of myosin assembly caused by changes in the level of heavy- or light-chain phosphorylation may be an important element in the pathway leading to the activation of cytokinesis⁸. But the protein may not need to assemble into filaments to translocate particles along actin filaments, a process which can be driven by myosin monomers^{9,10}. A new procedure for visualizing myosin filaments in cells⁸ could be used to determine whether phosphorylation regulates myosin filament assembly *in vivo* as well as *in vitro*.

How similar is thymus myosin to the protein from other tissues? Properties such as enzyme activity, immunological cross-reactivity, filament assembly and regulation of myosin isoenzymes differ, so thymus myosin could be a special case. Indeed, it differs from chicken gizzard smooth muscle myosin, where the major effect of light-chain phosphorylation is greatly to increase the V_{max} of the actin-activated ATPase activity¹¹, suggesting that phosphorylation stimulates the actin-activation of this myosin even at very high concentrations of actin. Phosphorylation of skeletal muscle myosin light chains has a much smaller effect on the actin-activated ATPase activity, causing only a twofold decrease in K_{app} and no change in V_{max} (ref. 12). It would be interesting to know whether these differences reflect physiologically significant differences between non-muscle, smooth and skeletal muscle contractile systems.

Another question concerns whether phosphorylation of myosin from thymus and from other vertebrate non-muscle tissues can regulate motility (as opposed to enzyme activity and filament formation). An assay for the movement of myosin-coated beads along actin filament bundles¹³ will be useful in addressing this question. Indeed, Sellers *et al.*¹⁴ have shown that light-chain phosphorylation regulates translocation of gizzard myosin-coated beads along actin filaments and have demonstrated a high correlation between motility and ATPase activity.

Some of the actin-binding proteins present in non-muscle cells may also contribute to the regulation of motility. Interestingly, Wagner and George² report that tropomyosin seems to mimic light-chain phosphorylation, causing a large decrease in the K_{app} of unphosphorylated thymus myosin to a level similar to the K_{app} of phosphorylated myosin. This suggests that unphosphorylated and phosphorylated myosin are equally effective in causing movement in regions of the cell that contain tropomyosin, even if the actin concentration is low. Actin filaments con-

taining tropomyosin plus troponin (a skeletal muscle Ca^{2+} -binding regulatory protein) activate the ATPase activity of unphosphorylated myosin in the presence, but not in the absence (less than 10^{-6}M) of Ca^{2+} .

In contrast, activation of the ATPase activity of phosphorylated thymus myosin by actin-troponin-tropomyosin is insensitive to calcium. Therefore, light-chain phosphorylation stimulates the actin-tropomyosin-troponin-activated ATPase activity of thymus myosin² independent of the actin concentration when the Ca^{2+} concentration is less than micromolar. But it is not known whether troponin is present in non-muscle cells.

To elucidate the functions of myosin in non-muscle cells, antibodies that specifically inhibit the actin-activated ATPase activity of myosin can be microinjected into living cells. Treatment of cells in this way causes an inhibition of cytokinesis but not mitosis^{15,16}.

Analogous experiments, using probes that specifically inhibit myosin phosphory-

lation in living cells (such as antibodies to myosin kinases) might similarly illuminate the role of this modification in the regulation of actomyosin-based motile activities in many cells. □

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Molecular biology

Brevity is the soul of wit

from Roger H. Pain

IN HIGHER organisms, protein-coding genes are made up of exons, each of which encodes relatively short sections of the protein chain, and introns, intervening regions of DNA that are not translated into protein. This revolutionary discovery led to speculation that this sort of gene structure could provide the basis for rapid and radical steps in the evolution of proteins¹. One development of this idea is the proposal that the sections of protein encoded by exons have particular biochemical functions within the whole molecule; in 1981 M. Gō assigned different functions of the haemoglobin molecule to the three exons of the globin gene². In the 5 March issue of the *Journal of Molecular Biology*, De Sanctis and colleagues report that a polypeptide corresponding closely to one of these exons binds haem to form a 'mini-myoglobin' which exhibits the oxygen-binding function of whole myoglobin³, providing experimental evidence for Gō's hypothesis.

The idea that one structural and functional domain of a protein is encoded by one exon has developed as the structures of more genes have become known. The function of a domain can range from specific antigen binding to the provision of small sections of peptide or 'beads' that can be strung together by exon multiplication to form a chain of a functionally appropriate length. But if the larger exons originally encoded 'ancestral' protein units (those that existed in isolation from

other exon-coded units), then the polypeptide must have been able to fold into a reasonably stable conformation to exercise the specific function on which its evolutionary selection would be based⁴.

Experimental confirmation of these proposals, in terms of the ability to fold and the expression of a biochemical function, is made difficult by the sophisticated and specialized mutational changes in the ancestral protein unit that have led to the present-day form of the protein. Changes in amino-acid composition that strengthen the interaction of the ancestral unit with other exon products, and which modify or supplement the original function, may decrease the ability of the original structure to function independently. Increasing the number of non-polar amino-acid residues on the surface of a protein, for example, can strengthen the interaction between two domains but at the same time make the isolated unit less stable in aqueous solution. Therefore, exon-coded domains may only be fossils, with no relevance to their intrinsic structure or function. But if it is true that, throughout evolutionary modification, the ability of the domains to fold into a very precise three-

dimensional conformation is retained or strengthened at all steps of development of each protein, an experimental test of the hypothesis should be possible.

The first protein structure to be determined in three dimensions was myoglobin. Watson and Kendrew showed that this protein consists largely of helical segments of backbone chain folded back on one another in such a way that one side of each helix points to the solvent and the other to the core of the molecule. A cleft in this structure, lined with appropriate amino-acid residues, receives the haem

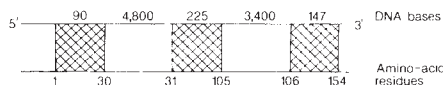


Fig. 1 The myoglobin gene of the grey seal⁷.

group like a coin entering a slot. The gene encoding myoglobin comprises three exons separated by long introns (Fig. 1). Gō performed a nearest-neighbour analysis of the amino-acid residues in the structure of globin and found that the peptides encoded by the two outer exons fold so as to fall naturally into two clusters (or domains) of amino acids, separate from each other and flanking the remaining peptide that folds to form the haem slot (Fig. 2). The bonds joining residues 30–31 and 105–106 each occur within helices, hence these exons do not encode neat domains that are immediately obvious to the eye, unlike those in immunoglobulins and nucleotide-binding enzymes.

In their new work, De Sanctis *et al.* excised a peptide from myoglobin that corresponds to that encoded by the central exon but which contains an additional 30 residues at the carboxy-terminal end. This peptide binds haem with high affinity and then behaves as a mini-myoglobin by combining reversibly both with carbon monoxide and with oxygen. Remarkably, the kinetics of these reactions are very similar to those of whole myoglobin.

These results provide strong evidence that the central exon encodes a functional unit, whose structure is very similar to that in the intact protein, in order that amino-acid residues that are essential for oxygen binding (such as histidines) may be oriented precisely towards the haem. A

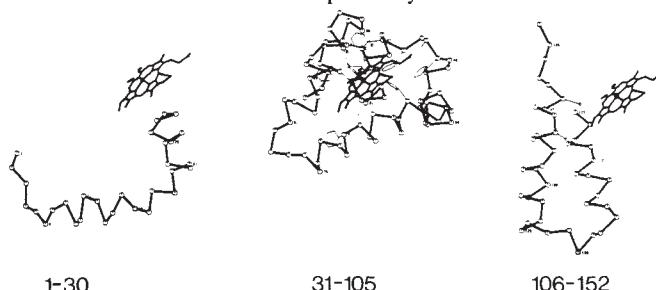


Fig. 2 Conformation of the three exon-coded peptides of the beta-chain of haemoglobin, which shares a common three-dimensional conformation with myoglobin². Numbers, amino-acid residues.

similar experiment using the precise central domain was only partly successful⁵, supporting the notion that, without additional stabilization, the domain of the present-day protein lacks the stability that is presumed to have existed in the ancestral exon product.

Despite the apparent evidence to the contrary from the globin gene structure, Gō proposed that the peptide encoded by the central exon comprises *two* structural domains². Subsequent analysis of the gene encoding plant leghaemoglobin, which shares the globin family conformation, showed the presence of four exons with the additional intron exactly where Gō predicted.

Has the break been gained recently, by

insertion⁶, or does this reflect the primordial gene structure? If the latter is true, the experiment of De Sanctis *et al.* suggests that a functional role for either or both of these domains could be assigned. Who will have the wit to explore the ultimate brevity of micro-myoglobin? □

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Topology

The Poincaré conjecture proved

from Ian Stewart

A SOLUTION to the Poincaré conjecture, one of topology's biggest open questions which dates from 1904, was announced earlier this month by Eduardo Rêgo (Centro Matemática Ruy Luis Gomez, University of Oporto) and Colin Rourke (Mathematics Institute, University of Warwick). Their work sets the seal on some 80 years of intensive effort by mathematicians, and represents a fundamental advance in our understanding of three-dimensional topology.

Topology is the study of those properties of objects that are unchanged by continuous transformations. Its origins can be traced back to 1735 when Leonhard Euler solved the problem of the Königsberg bridges, but it first acquired mathematical significance around 1860 with the work of Georg Bernhard Riemann on Riemann surfaces. Over the past 50 years it has developed into a major area of mathematics, and its influence now extends throughout the subject. It is now starting to play an important role in applied science; for example there are topological questions in the same general area as the Poincaré conjecture that lie at the heart of current attempts to unify the fundamental forces of nature.

A major triumph of topology in the nineteenth century was the complete classification of (closed compact) surfaces. The orientable, or two-sided, surfaces are all equivalent to a sphere or a torus with one or more holes. (The number of holes is called the genus of the surface.) The non-orientable, or one-sided, surfaces are similarly classified by their genus. The genus is a topological invariant: if one surface can be transformed continuously into another, the number of holes does not change. This implies that all possible surfaces may be topologically classified by

just two items of data: orientability/non-orientability; and the number of holes.

One of the most important problems in topology is to extend these classical results on surfaces to their multidimensional analogues, called manifolds. (A surface is a two-dimensional manifold.) For example, it would be extremely useful to have a complete classification of (closed compact) manifolds of any given dimension. The main idea is to associate with each manifold various algebraic structures that capture key features of the topology. One of the most basic, introduced by Henri Poincaré in 1895, is the fundamental group. This determines the way in which closed loops in a manifold may be deformed into one another.

For example, consider a circle, the simplest one-dimensional manifold. Two loops round a circle may be deformed into each other if, and only if, they wind round the circle the same number of times. (Topologists then call them homotopic, meaning similarly situated.) The algebraic structure arises because loops can be 'added' by joining them end to end. If loops with winding numbers m and n are so joined, the result winds round $m+n$ times. The fundamental group is thus the set of integers (representing the winding numbers) under the operation of addition (representing the joining of two loops in sequence).

For a two-dimensional sphere — the surface of a solid ball — the fundamental group is trivial, that is, it consists of a single element. This happens because any loop on a sphere may be shrunk to a point by sliding it over the sphere. The two-dimensional sphere is said to be simply connected. For this and other reasons topologists consider the two-sphere to be the simplest of all surfaces, that is, the one

with fewest topological complications.

For a torus (or doughnut) two distinct winding numbers are required: the first representing the number of times the curve winds around the hole in the middle; the second the number of times it winds around the body of the torus (like the stripes on a barber's pole). The fundamental group therefore consists of pairs (m, n) of integers, and the operation of joining loops again corresponds to arithmetical addition: $(m, n) + (p, q) = (m+p, n+q)$. As this is a different algebraic object from the integers or the trivial group, it follows that a torus cannot be topologically deformed into a circle or a sphere. That a torus is not topologically equivalent to a circle is obvious on other grounds; that it is not deformable into a sphere is less obvious. For more complicated examples, the fundamental group plays a central role.

The fundamental groups of all surfaces can be calculated, and it can be seen that they are all non-trivial (that is, they contain loops that cannot be shrunk to a point), unless the surface is a two-sphere. In other words, any surface with a trivial fundamental group must be topologically equivalent to the two-sphere.

The Poincaré conjecture is the exact analogue of this fact for the next dimension of manifolds — three-dimensional manifolds. Once again there is a particularly simple three-dimensional manifold, the three-dimensional sphere, which can be thought of as ordinary three-dimensional space, but 'gathered together' at infinity so that it closes up on itself, much as a square of cloth can be gathered into a closed bag by pulling tight a loop of string around its edge. The three-sphere has a trivial fundamental group (every loop can be shrunk to a point). Poincaré's question (*Rendiconti del Circolo matematico di Palermo* **18**, 45; 1904, reprinted in *Oeuvres de Henri Poincaré* Vol. VI p.498; Gauthier-Villars, Paris, 1953), posed with the off-hand remark "this question would lead us too far astray", is the converse. If a (connected closed compact) three-dimensional manifold has a trivial fundamental group, then must it be topologically equivalent to the three-sphere? Poincaré's problem turned out to be one of the most baffling in mathematics, and it soon joined the ranks of other notorious and difficult unsolved problems, such as Fermat's last theorem or the Riemann hypothesis.

To explain why the problem turned out to be so difficult, and to explain how Rêgo and Rourke have now solved it, we need to look at the higher-dimensional analogues of the problem. The fundamental group, using as it does one-dimensional loops, is incapable of capturing certain features of higher-dimensional manifolds. For example it cannot distinguish between a solid ball and its two-sphere boundary.

But analogous invariants may be constructed using 'loops' (actually spheres) of higher dimension. These objects are called the higher homotopy groups. In the subject of algebraic topology, many variations on this theme have been introduced, calculated and analysed. A major part of the research effort of the past 50 years has gone into refining such ideas.

It can be shown that if a three-dimensional manifold has a trivial fundamental group, then it is a homotopy three-sphere: that is, all of its higher homotopy groups (fundamental and higher) are the same as those of the three-sphere. Poincaré's problem now has an immediate generalization to all dimensions: must every homotopy sphere be a genuine sphere?

Ever since Poincaré stated his problem, it and its generalization have been under incessant (and largely unsuccessful) attack. Only for the two-dimensional case — surfaces — was the answer known to be 'yes', as a result of the classification theorem. While topology grew ever more powerful, Poincaré's problem remained almost totally intractable. It is not hard to see why. To calculate the higher homotopy groups of a given manifold is itself a difficult task; but the Poincaré conjecture asks the inverse question: given the groups, find the manifold. To prove it, one must start with a completely arbitrary manifold, and then somehow use the information on the higher homotopy groups to bring it into a recognizable form. For surfaces, a case-by-case analysis will do the trick, because the classification theorem lists all the cases. But in three dimensions or more, no classification is known, and to make matters worse, the unknown status of the Poincaré conjecture is to some extent responsible for that state of affairs. There is an air of Catch-22: before proving the Poincaré conjecture, one must first prove the Poincaré conjecture. The only way out is to develop totally new methods and bypass the unsolved problems of classification.

The first big breakthrough came in 1961 when Stephen Smale developed a method for breaking manifolds into nice pieces, called handles. By moving handles around he proved a far-reaching theorem, for dimensions five or higher, one of whose consequences is the truth of the Poincaré conjecture for dimensions $n \geq 5$. Originally, Smale's proof worked only for $n \geq 7$. John Stallings dealt with $n=6$ and Christopher Zeeman with $n=5$. Smale subsequently extended his methods to these dimensions.

Another important tool for the study of higher dimensional manifolds was also developed in the early 1960s — the technique of surgery. This is in essence a systematic study of the effect of removing certain nice pieces of a manifold and gluing them back with a specific 'twist'.

Using surgery, John Milnor found a counterexample in dimension seven to the analogue of the Poincaré conjecture for a more specialized case. This is the 'differentiable' case where the topological equivalence is required to be differentiable as well as continuous. Milnor and Michael Kervaire obtained a complete understanding of this more specialized formulation of the conjecture in dimensions five and higher.

That left only the three-dimensional case (Poincaré's original problem) and the four-dimensional case unsolved; but here both handle theory and surgery fail to work. The number of dimensions is high enough to allow complicated behaviour, but too small to leave adequate room for manoeuvre in eliminating those complications. The general feeling was that something unusual happens in spaces of dimension three or four. But in the late 1970s the Cambridge mathematician Andrew Casson found a way to make handle theory start to work in four dimensions. In 1982 Michael Freedman used 'Casson-handles' to prove the four-dimensional case of the Poincaré conjecture (but not the more specialized differentiable case, which remains open).

This left just one case of the problem unsolved — the dimension three problem which Poincaré had originally posed! Here the more specialized differentiable case is known to have the same answer as the continuous case. It is this final question that has now been answered, affirmatively, by Régis and Rourke. Their results have been written up as three University of Warwick preprints (*A Characterization of Homotopy 3-Spheres I and II*; and *Characterizations of S*). The proof has been subjected to detailed scrutiny by several experts and no errors have yet been found; it was discussed at a special meeting in low-dimensional topology held at Warwick this month. Although it is wise to be cautious, it seems that the problem really has been solved at last.

The new proof is based on an ingenious combination of both methods used to solve the higher-dimensional problems — handle theory and surgery. In dimension three, handle theory was already a well-established tool dating back to the diagrams devised by the nineteenth century mathematician P. Heegaard. But despite many intensive efforts, little progress had been made in using Heegaard theory to prove the Poincaré conjecture. Surgery, on the other hand, was not exploited systematically in the analysis of three-dimensional manifolds until Robion Kirby introduced his 'Kirby calculus' in the 1970s (*Inventiones Mathematicae* 45, 35; 1979). The surgery description of a three-dimensional manifold can be seen as a complicated picture of linked curves. Unfortunately many distinct forms of the links can correspond to the same mani-

fold. The Kirby calculus attempts to deal with this difficulty by giving a complete list of 'moves' that can be made in the link picture, without altering the manifold obtained by surgery.

In 1977 Joan Birman and W. Powell observed that there is a useful class of surgery descriptions that corresponds at once to Heegaard decompositions. It is this class of surgery descriptions that is exploited by Régis and Rourke. They consider simultaneously three descriptions of a homotopy three-sphere. These comprise a surgery description of the Birman-Powell type in the standard three-sphere; the corresponding Heegaard decomposition; and the dual surgery description in the homotopy three-sphere. Régis and Rourke introduce 'moves' that alter all three descriptions in recognizable and predictable ways, and by using these moves they rearrange the three descriptions until the one based on handles — the Heegaard decomposition — is finally rendered accessible to the ordinary handle moves used by Smale. At this point the homotopy three-sphere is seen to be equivalent to the standard three-sphere.

The simultaneous moves of Régis and Rourke can be viewed either as complicated sequences of Smale moves, or as complicated sequences of Kirby moves and their duals. In fact they are related to these more elementary moves in approximately the same way that the complicated sequences of twists used to unscramble a Rubik cube are related to the elementary twists of which they are composed. (This is a loose analogy only; the Rubik cube is not relevant to the problem.)

Ironically, the work of Régis and Rourke goes against the prevailing wisdom. The main focus of recent work on three-manifolds has been directed towards the remarkable discovery by William Thurston of connections between three-manifolds and non-Euclidean geometry. One hoped-for end-result of Thurston's programme was to have been a proof of the Poincaré conjecture. The 'classical methods' of Heegaard decompositions or the Kirby calculus had been almost written off as too unsophisticated to tackle the Poincaré conjecture.

The results of Régis and Rourke put the finishing touches on many known results in topology, by removing unnecessary hypotheses. There are also important consequences in four-dimensional topology, particularly the existence of a four-dimensional manifold having no triangulation. And their pioneering effort paves the way, at last, for a detailed understanding not just of the three-sphere, but of three-manifolds in general. Of course the book of topology is by no means closed; but a major chapter is. □

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Origins of human T-lymphotropic viruses

SIR—Joseph Rosenior finds it hard to understand why we have postulated that human T lymphotropic virus (HTLV-I) originated in Africa¹. We were surprised by his belief that Japan did not have contact with Europeans until the eighteenth century. While it is true that "Japan was virtually cut off economically and politically from the rest of the world", it is striking that the initial contacts with Portuguese explorers during the sixteenth century occurred precisely in areas of Southern Japan where HTLV-I is endemic. The first Portuguese contact arrived in 1543². In 1549, Saint Francisco Xavier, a Jesuit missionary, arrived in Kagoshima. He later gained Lord Omura Sumita's sanction to use the Port of Nagasaki. This port prospered and became a large commercial centre. The Portuguese established themselves throughout the southern portion of Japan and their influence and contact with the Japanese became frequent. It is known that the Portuguese took Africans with them to Japan. The Africans' presence can be seen in Portuguese pictorial records known as Nanban-Byobu, where the artist depicted Portuguese, Japanese and Africans together^{3,4}.

Further, it is believed that the Portuguese came with African monkeys. The Japanese word for monkey *amakawa* is thought by Juijirô Cogô to be derived from the Portuguese word *macaco* also meaning monkey (ref. 5 and p.92, ref. 1).

Recently, Hino *et al.* supported this conclusion by demonstrating that the proportion of HTLV-I disease was correlated with the incidence of Japanese Catholics⁶, again consistent with this hypothesis. A virus similar to HTLV-I was found in Japanese macaques, stimulating an alternative idea that HTLV-I entered Japan from a monkey. However, viruses like HTLV-I were also found in many African monkeys⁷ and Yoshida, Seiki and colleagues recently found that the African monkey virus (STLV-I) is almost identical to the HTLV-I in man (African and Japanese)⁸.

Rosenior is also surprised that we think that HTLV-III, the virus which causes AIDS, also originated in Africa. He states "that HTLV-III occurs . . . in some flocks of European sheep." It is ironic that he refers to our own work (with M. Gonda), although he has misinterpreted the results. Visna virus of sheep is *very distantly* related to HTLV-III. Both viruses probably have a very ancient ancestral origin in common. HTLV-III is much more closely related to the simian virus STLV-III, the virus isolated by Essex and colleagues from African green monkeys. All current data from HTLV-I and HTLV-III support an African origin for these two human

retroviruses. Just as important, no alternative reasonable hypothesis has been presented.

Finally, the origin of the only other human retrovirus, HTLV-II, is unknown, but its relatedness (about 50% of the genome) to HTLV-I suggests that HTLV-II and HTLV-I have common ancestry.

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Bovine leukaemia virus and multiple sclerosis

SIR—Koprowski *et al.* provide evidence that the serum and spinal fluid of multiple sclerosis patients contain antibody which cross-reacts with human T-cell viruses and that cells from the spinal fluid contain RNA which hybridizes with human T-lymphotropic virus (HTLV) type I under low-stringency conditions¹. But is it possible that the 'footprints' which they have found are actually those of bovine leukaemia virus (BLV)?

There are strong geographic correlations between production and consumption of dairy products and multiple sclerosis². Moreover, milk from cows shedding BLV has caused leukaemia in chimpanzees³ and there is epidemiological evidence linking cattle herds infected by this virus with human leukaemia⁴. Therefore, it seems likely that BLV can be transmitted in dairy products and cause disease in humans.

It also seems likely that the viral traces found in multiple sclerosis patients could have been left by BLV. Antisera to BLV proteins cross-react with those of human T-cell viruses. Amino acid microsequencing and statistical analysis reveal 9 common amino acid residues out of 24 possible comparisons when the NH₂-terminal sequences from the core proteins of HTLV p24 are aligned. The probability

that this sequence homology is due to chance is only 0.4×10^{-10} (ref. 5). Nucleic acid hybridization experiments thus far reveal a sequence homology of 11% between the RNA of BLV and HTLV⁶.

Diets low in saturated fats (with sharp reduction in the use of dairy products) are said to help patients with multiple sclerosis⁷ and the Guillain-Barré syndrome⁸. It is an interesting coincidence that one of the control subjects studied by Koprowski *et al.* was a patient with Guillain-Barré syndrome who also had antibody to HTLV.

In the aggregate, these facts should prompt some consideration of BLV infection in patients with multiple sclerosis and, perhaps, certain other neurological syndromes of unknown cause.

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Endogenous retrovirus in multiple sclerosis?

SIR—In their recent article¹ Koprowski *et al.* described the presence, in sera from some patients suffering from multiple sclerosis, of antibodies reactive with antigens of the human T-lymphotropic viruses HTLV-I, -II and -III. Although the authors did not distinguish between healthy blood donors possessing no anti-viral antibodies or those possessing antibodies only occasionally in lower titres (there was no definition of cut-off in the enzyme-linked immunosorbent assay), they cautiously suggested that infection by an exogenous, so far unknown retrovirus strain might be involved in the development of multiple sclerosis. The induction of antibodies that cross-react with such immunologically divergent strains as HTLV-I and -III and the inhibition by both HTLV-I and -III of antibody binding to HTLV-I p24 suggest that the putative multiple sclerosis strain must be very different from the two strains investigated. Testing of sera from multiple sclerosis patients against a variety of available animal retrovirus antigens may help to predict more precisely the antigenic composition of a putative multiple sclerosis retrovirus.

However, there is an alternative explanation for the data reported, namely the possible activation of a human endogenous retrovirus in cells of multiple sclerosis patients either directly by infection with viruses (such as measles, varicella, influenza) often associated with

the development of multiple sclerosis, or indirectly and more likely, by immunological response to such virus infected cells. It is well known that chromosomally integrated, endogenous or exogenous retroviruses can be induced to replicate by immunological mechanisms.

Evidence for the existence of a human endogenous retrovirus is slowly, but steadily accumulating. Retrovirus-like particles can regularly be observed budding from human placental trophoblasts (see ref.2 for review). Morphologically indistinguishable viruses have been detected in all investigated trophoblast-containing human teratocarcinoma cell lines. Animal retrovirus core (gag-) related antigens and corresponding antibodies have repeatedly been found in healthy individuals and in patients with autoimmune disorders (most recently in ref.4). Furthermore, naturally occurring antibodies to endogenous retroviruses are widespread in animals, including higher primates⁵.

Like exogenous retroviruses, induced endogenous strains insert their envelope glycoproteins into the host's cell membrane, rendering the cell vulnerable to immunological attack⁶. In astrocytes or other glial cells, this process may lead to immunological exposure of myelin basic protein (MBP) or other antigens, sensitizing and activating MBP-specific T cells. Thus, induction of endogenous retroviruses, either directly by horizontally transmissible viruses like measles, varicella or influenza or indirectly by the immune response to such virus infected cells, may turn out to explain anti-retroviral antibodies in multiple sclerosis patients.

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Archaeobacterial status quo is defended

SIR—In the interest of the young progressive field of molecular phylogeny the debate between Lake and the "orthodoxy of archaeobacteria" should be reduced to its factual basis. Lake *et al.* claim (1) that the urkingdom of the archaeobacteria¹⁻³ be divided into two distinct urkingdoms — the "eocytes", comprising the genus *Sulfolobus* and the Thermoproteales and the "archaeobacteria", comprising the rest⁴; and (2) that the Halobacteriaceae (or, rather, Halobacteriales) be transferred from the urkingdom of the archaeobacteria into that of the eubacteria which they, after its expansion, re-christened "photocytes"⁵.

• Lake bases his claims on a comparison

of details of the three-dimensional structure of ribosomes as revealed by electron microscopy. The phylogenetic usefulness of this feature, at the present level of resolution, has been profoundly queried by Stöffler-Meilicke *et al.* and Stöffler and Stöffler-Meilicke⁷.

• The division of the archaeobacteria into two branches, methanogens plus halophiles and sulphur-dependent archaeobacteria, had been recognized previously^{8,9}. Yet the sequences of 16S rRNAs^{10,11} and many other feature designs of both branches are clearly too similar to justify their promotion to kingdom level¹⁰.

• The alleged homology of the purple membrane of some halobacteria and the photosynthetic machinery of several groups of eubacteria, the only argument besides ribosome shape quoted in support of the creation of the "photocyte" kingdom, is at best an analogy in principle (light energy utilization). Homology (or identity of origin) must be proven before it can prove relatedness.

Lake *et al.* discount the features supporting the "orthodox" view of one kingdom of the archaeobacteria including the halophiles mainly in two ways:

• So-called plesiomorphic properties (shared by "archaeobacteria", "eocytes" and Halobacteriaceae but not eubacteria) are considered useless for cladistics in contrast to the "synapomorphic properties" which have served to establish the proposed tree. The reason for this astonishing conversion of good archaeobacterial features ("orthodox" meaning) into "shared" plesiomorphic properties is simply the proposed division of one phylum into three. Are there other "synapomorphic" features common to halobacteria and eubacteria? And, if the archaeobacterial properties are plesiomorphic, why are they not shared by any eubacteria? Such terms should only be assigned after analysis and not used in a dialectical and tendentious way.

• As Lederer explains below, the proposals of Lake *et al.* in contrast to the tree derived from rRNA sequences comparison, prove invalid when tested by the consistency criteria of Felsenstein¹². The kingdom of the archaeobacteria remains a solid entity in our incomplete understanding of the early phase of biotic evolution.

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SIR—In his letter "An alternative to archaeobacterial dogma" (*Nature* **319**, 626; 1986), Lake claims that the archaeobacterial tree proposed by Woese and Olsen (*System. appl. Microbiol.* in the press) is inconsistent because of unequal clock-rates in different branches of the tree, citing the work of Felsenstein (*Syst. Zool.* **27**, 401; 1978).

According to Felsenstein, in an unrooted tree with four branches the relevant sums of transition probabilities, which determine the branching of the tree, are not affected by the exact placement of the root and thus different clock-rates.

By alternatively omitting one branch of the two trees shown in Lake's letter, five trees with four branches are obtained in both cases. Taking the branch lengths to represent the transition probabilities, one can calculate Felsenstein's probability sums ($P_{1100} + P_{0111} + P_{1010} + P_{0101} + P_{1001} + P_{0110}$). All five trees with four branches deduced from the tree of Woese did not violate the consistency condition ($P_{1100} + P_{0111}$ must be largest), whereas the five trees deduced from the tree of Lake violated the consistency condition in four out of five cases. In the one consistent case (a tree with eukaryotes omitted), the phylogenetic distance between methanogens and halobacteria used in Lake's tree was 30% greater than that found in Woese's tree.

Using Felsenstein's criteria it therefore seems inappropriate to depart from the "archaeobacterial dogma".

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Beyond hope: Bohr and physics

R.H. Dalitz

Niels Bohr: A Centenary Volume. Edited by A.P. French and P.J. Kennedy.
Harvard University Press:1985. Pp.403. \$27.50, £16.50 (£25.25 from 1 April).

NIELS BOHR was born on 7 October 1885; he died on 18 November 1962. His centennial year was the occasion for many meetings and addresses about his life and work, and its impact on science today. This collection of about 40 articles gives a broad coverage of these topics, together with a chronology, a full publication list and a substantial glossary (17 pages), all at a quite modest price.

It is a chastening experience for those of the present generation to look back into history and see how many parts Bohr played and how pervasive was his influence on physics in the twentieth century. Most of us, for example, know little of the genesis of Bohr's atomic theory, beyond the brief mention of the Bohr atom in today's textbooks. To follow his original arguments is a most instructive experience, and in the book J.L. Heilbron brings out well the tenacious way Bohr explored ideas, testing them in a flexible framework of physical principles which were in the course of becoming established. No wonder that Bohr's collaborator J.C. Slater could see him as "a remorseless fanatic" who persisted in pressing on beyond hope, until some link with other truths emerged or that line of thought became decisively excluded. He sought continually to link up half-formed ideas, building upon dimly glimpsed relationships. His correspondence principle was one such relationship, and very much his own — more a way of life than a principle, said some. In his contribution, H. Kragh traces out the steps Bohr took to achieve his semiclassical

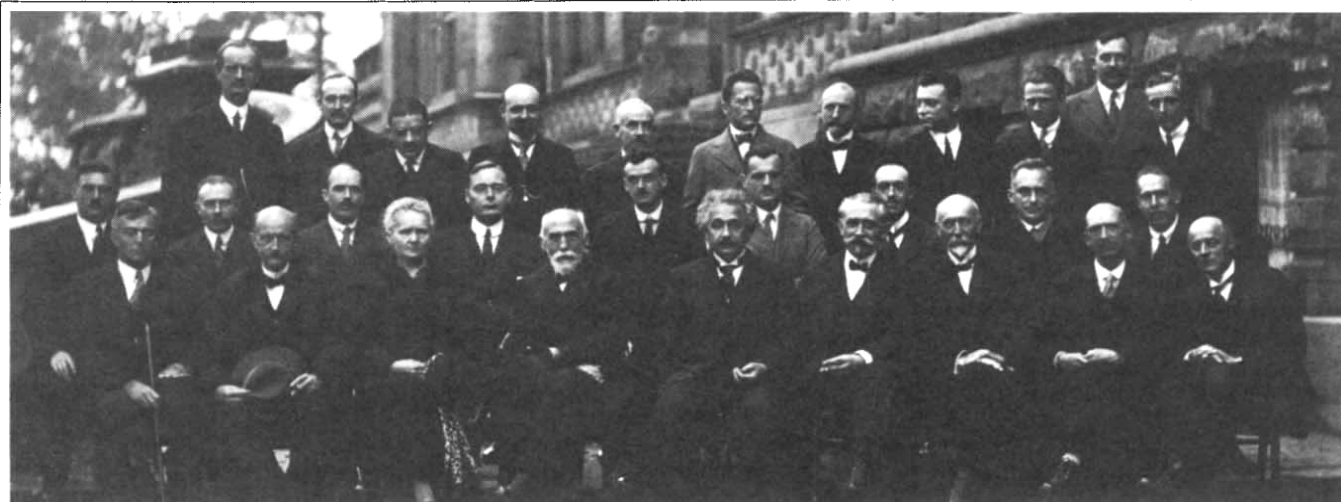
theory of the periodic table, a product of great intuition and completed long before Pauli's exclusion principle.

Bohr's association with Rutherford was vital in this early period, not only because Rutherford's laboratory in Manchester was the centre for the exploration of nuclear aspects of atoms and molecules but also because of the fruitful interaction between their personalities. As Bohr worked and worked, waiting to convince himself that his atomic model covered the whole periodic table, Rutherford finally thundered "You explain hydrogen, you explain helium, and everybody will believe all the rest". And so Bohr's "Trilogy" of papers emerged, forwarded by Rutherford to the *Philosophical Magazine*, published there in 1913 and rewarded by the Nobel Prize for 1922.

A second phase of Bohr's life began with his appointment to a professorship in the University of Copenhagen in 1916 and blossomed when his Institute of Theoretical Physics was set up in 1921. This quickly became a Mecca for physicists from around the world. Bohr was readily accessible to all who went there, and years spent in Copenhagen left an indelible mark on those who had the good fortune to visit the Institute. Bohr continued to work on his atomic theory; element 78 was discovered there through its guidance and named hafnium, after the Latin for Copenhagen. The well-known "BKS" paper (with Kramers and Slater) was written there in 1923, but despite Compton's direct evidence for "light quanta" Bohr

was unwilling to relate his atomic transitions to the concept — this paper did not accept them as particles and implied that energy and momentum conservation were only statistical, as is recalled here in Slater's article. However, Heisenberg soon arrived at the ideas and relationships which quickly became "matrix-mechanics"; concepts and theoretical ideas developed rapidly at this point, stimulated by Dirac's interpretation of Heisenberg's non-commuting matrices as dynamical operators and Schrödinger's wave-function, with its probabilistic interpretation. Heisenberg's uncertainty relationship demonstrated that position and momentum could not both be known precisely, and this led Bohr to his notion of *complementarity*, a logical relationship required to express mutual exclusion between two physical quantities observable by different kinds of measurement on the same physical system.

Although holding much truth, complementarity has always been rather ill-defined. The eventual upshot was the "Copenhagen interpretation" of quantum mechanics, which holds the measuring apparatus to be an essential part of the system in the process of observation, and which Bohr used in a disarming way. For example, the dramatic discussions between Bohr and Einstein at the Solvay Conferences of 1927 and 1930 are here recalled by long extracts from Bohr's own account of these breakfast-time dialogues. Each morning, Einstein would propose a new paradox to confound quantum theory, which Bohr was able to meet at the end of the day by showing that the quantum uncertainties inherent in the measuring devices were just sufficient to annul Einstein's contradiction. These sequences of challenge and riposte represent one of the most remarkable episodes in the history of scientific thought; although Einstein was not ever convinced, Bohr's success



The Solvay Conference of 1927, held in Brussels between the 23rd and 29th of October. As well as Bohr, among those present in the photograph are H.A. Kramers, P.A.M. Dirac, W. Heisenberg, E. Schrödinger, A. Einstein and W. Pauli.

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with the Copenhagen interpretation brought it widespread acceptance.

In 1935, "a bolt from the blue" arrived in the form of the Einstein-Podolsky-Rosen (EPR) paradox, which apparently showed a violation of causality by quantum mechanics and was, in the authors' view, in conflict with everyday experience ("EPR reality"). This paradox and its later "delayed-choice" elaborations are discussed in the centenary volume by V.D. Mermin and P.J. Kennedy, respectively. Today we know empirically that quantum coherence exists on a macroscopic scale, not only at the atomic level. Laboratory experiments verifying the quantum mechanical result questioned by EPR are now carried out over metres, so that these phenomena must now be regarded as part of our everyday reality. In other words, the "EPR paradox" was simply due to the incompleteness of "EPR reality", which did not embrace the fact of quantum coherence in our everyday world. Of course, most of us still find such coherence an almost incredible fact, but this is only a reflection of our personal limitations. We need another Bohr to reshape our way of looking at things, but probably the next generation of physicists will see no difficulty in all of this.

With the discovery of the neutron in 1932, and the early exploration of nuclear reaction processes, Bohr was drawn into the problems of nuclear physics, the third phase of his life, covered here mainly by R.H. Steuwer. Bohr's contributions — simple, convincing and deep — were typical of the man. He related the sharpness observed for nuclear levels excited by slow neutrons to the many degrees of freedom of the nucleons within a nucleus, illustrating this by a wooden model with balls (nucleons) lying in a depression (the nuclear well). This led him naturally to the idea of "the compound nucleus" as an essential intermediate state in all nuclear processes. However, Bohr over-estimated its range of validity and used it to discourage speculations about nuclear shell-models, thereby delaying their development by two decades. At high energies, nuclear matter proved to be far less opaque to nucleons than he guessed; even at intermediate energy, many important nuclear processes do not proceed through a compound nucleus. Bohr understood at once the implications of the Hahn-Meitner discovery of nuclear fission in 1938, also realizing that the slow-neutron fission they observed was due to capture by the isotope ^{235}U . With J.A. Wheeler, he then began a major calculation of the mechanism of nuclear fission, based on the liquid drop model of the nucleus, and by December 1939 was already speaking publicly of the possible liberation of nuclear energy on a practical scale.

Bohr allowed the possibility that every next step might require some quite revolu-

tionary idea. His reply to a far-out idea proposed tentatively by Pauli was characteristic: "Yes, but unfortunately it is not crazy enough". Twice he proposed energy nonconservation as necessary to explain some new phenomenon, quickly withdrawing the idea as soon as some less revolutionary explanation was seen to be adequate (for example, when Pauli's neutrino hypothesis predicted the beta-decay spectrum observed).

In a 1932 lecture, "Light and Life", reprinted in the book, Bohr expressed some unusual ideas about biology which he did not qualify until 1962. Life was an additional mystery, he implied; measurement might kill a living system and this new factor might require an extension of complementarity in biology, and some modification of the physical laws acting there. Bohr's views motivated more than one physicist (M. Delbrück and G.S. Stent, certainly) to move on to biological problems*; ex-physicists did indeed cause a revolution in molecular biology, although not for the reasons foreseen by Bohr. Stent (*Science* **160**, 390; 1968) has re-examined Bohr's early views (apparently unaware of Bohr's 1962 qualifications of them) in the light of the later history of biology, concluding that no hint of phenomena inexplicable within the established physical laws has ever emerged.

Late in 1943, Bohr joined the atomic bomb project at Los Alamos because he felt that mankind faced a crucial challenge, where failure would lead to an appalling step backwards. Since the project was then far advanced, he concerned himself primarily with its long-term political implications. He sought to discuss these with the political leaders in Britain and the United States, but without much effect. Churchill regarded him as positively dangerous, for Churchill's concern was still to win the War, not to contemplate future problems, as M. Gowing and R.V. Jones recount. In this last, political, phase of his life, Bohr tried again in 1950, with his "Open Letter to the United Nations" which is reprinted in the book. The timing, however, was unfortunate; the Korean War broke out a few days later and the world paid little attention to it.

This volume brings together accounts of many different aspects of Bohr and his work in a coherent way, presenting them attractively, with many interesting photographs. It is much enlivened by the extracts of material from various sources which appear in the large margins of its pages. Altogether, a wide audience will find it to be a very stimulating book. □

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* A biography of Delbrück, by Peter Fisher, will be reviewed in *Nature* on 17 April.

Inside the workings of the brain

Oliver Braddick

Functions of the Brain. Edited by Clive Warwick Coen. Clarendon:1985. Pp.209. £15.

"THE brain is quite simply the most complicated object known." In a well-turned introduction, Clive Warwick Coen points out that research on this complicated object is in an exuberant but disorderly state. People from heterogeneous scientific backgrounds are simultaneously at work, using a variety of techniques to address multiple levels of explanation that have a habit of spilling over into each other. The brain itself has a diversity of functions, ranging from thermoregulation to planning sentences, which does not map at all tidily on to the divisions between scientific disciplines or their different methods of study. It follows that in planning a lecture series to provide an overview of current brain research, and assembling it into a book, it would be difficult and probably misleading to seek an ordered unfolding of some intellectual ground-plan. Better to arrange a kind of scientific pot-luck supper, to which a set of enthusiastic cooks each brings his own dish, be it spicy or subtle, a filling casserole or a fluffy confection. *Functions of the Brain* provides quite a satisfying buffet of this kind; the essays in it have some division by the brain functions they consider, but also illustrate their authors' very different scientific methods, objectives and styles.

H.B. Barlow's elegant discourse on perception illustrates how this area of neuroscience has come closest to possessing mature theoretical and experimental subsections of the sort that a physicist would recognize. Barlow argues that the visual system transmits signals about patterns of light in ways which optimize the efficiency of these neural representations; this helps us to understand the known forms of neural codes and their development in terms of the statistical properties of the visual environment, and it suggests principles of cortical organization which have yet to be experimentally tested.

Patrick Wall, who writes about pain, similarly uses his essay to develop a particular line of argument. He challenges what he characterizes as the dualist view, that deterministic sensory pathways supply information to a distinct, conscious central entity. His challenge comes from evidence that the subjective quality of painful sensation has a relation to events in sensory nerves that is very far from one-to-one, and that this is a consequence of the intimate two-way interaction of peripheral and central events in the ner-

vous system. Wall's path through this argument, and his view of its historical context, is more discursive and less focused than Barlow's, but his support of his philosophical approach by discussions of hypnosis, questionnaires in casualty clinics and President Reagan's gunshot wound is likely to intrigue a body of non-technical readers.

J.F. Stein's chapter on control of movement has a less personal tone than either of these two, but does give a clear account of the relations between structure and function in the multiple levels of motor control. Moreover, it provides a nice mirror image of Wall's message by emphasizing that, just as sensory processing is not a one-way flow of information from periphery to centre, motor actions cannot be understood as the hierarchical execution, via lower level reflexes, of centrally enunciated neural commands.

Barlow, Wall and Stein represent different facets of a tradition which, while based in detailed analysis at the neuronal level, likes to muse on the large-scale organizing principles of brain function. The molecular neurobiology of Craig Bailey and Eric Kandel has a much stronger smell of the laboratory bench, where there is little time to look back at intellectual history given the wealth of information that comes tumbling from new electrophysiological and ultrastructural techniques, and where an invertebrate lies waiting whose neuronal interconnections can be mapped *completely* by scientists with sufficient energy and insight. Bailey and Kandel's work on *Aplysia* brings together the global phenomena of habituation and conditioning with events occurring within identifiable synapses. We do not yet know whether the impressive ability to unite understanding of these levels in a simple organism will help in leaping the much more daunting chasms between subcellular and behavioural phenomena in higher vertebrates; students of mice and men must watch this space in anticipation.

George Fink, on neuroendocrine function, also has a molecular perspective, and like Bailey and Kandel he presents a good deal of experimental detail. Unfortunately he does not have as tightly concentrated a scientific story to tell, and so the non-specialist reader is likely to be dazed rather than carried forward by his table of 24 neuropeptides or his illustrations of the homologies in their amino-acid sequences. However, one compelling impression that remains, in particular from the figures in this chapter, is of the stunning armoury of techniques which the histochemically sophisticated researcher can now draw upon to look inside the nervous system.

Norman Geschwind's chapter represents yet another tradition, the oldest in the study of the brain, and one which he

eloquently defends. His recent death deprived us of one in the line of literate and audacious neurologists who extend their close and purposeful clinical observations into imaginative views of brain function. On occasion the breadth of Geschwind's narrative, offering keys to dyslexia, emotional expression and creative impulses, may sweep the reader over issues where those raised in more experimental traditions might want to pause and quibble. However, the approach of clinical neurology, which has recently been married with great effect to the systematic testing methods of cognitive neuropsychology, offers at present almost the only route for linking neural mechanisms with the specially human abilities of language, reasoning and spatial representation.

The chapters on specific topics are topped and tailed by essays from J.Z. Young and Colin Blakemore, respectively, both of whom adopt a more contemplative and philosophical stance. The issues they address — Young on the place of brain function in our broader view of life, Blakemore on what kind of explanation brain scientists should look for — are significant, yet neither really carries the sense of scientific mastery and clarity that marks the best of the other chapters (or, indeed, that can mark these authors when expounding their own work).

Not every active branch of brain research is well portrayed in the book. I would have welcomed more on the rich advances stemming from the combination of sophisticated behavioural techniques and electrophysiological recording in alert (and even mobile) animals, and from the renaissance in the anatomical study of cerebral pathways. Developmental neuroscience is also poorly represented. There is, too, understandable variation in the accessibility of the contributions: some (Wall's and Geschwind's, and perhaps Barlow's) can be read by the layman; others (for example Stein's, and Bailey and Kandel's) are heavier on technical detail and will be of more benefit to those in adjacent disciplines. Nonetheless *Functions of the Brain* is a creditable and often stimulating sampler of what we know — and don't know — about the brain. □

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New in paperback

- *Princes and Peasants: Smallpox in History*, by Donald R. Hopkins. Publisher is University of Chicago Press, price is \$12.95, £10.95. For review see *Nature* 310, 608 (1984).
- *Wandering Lands and Animals: The Story of Continental Drift and Animal Populations*, by Edwin H. Colbert, which first appeared in 1973. Publisher is Dover, New York/Constable, London, price is \$7.95, £7.55.

Ada and the engines

Richard Gregory

Ada: A Life and a Legacy. By Dorothy Stein. MIT Press:1985. Pp.321. \$19.95, £17.50.

ADA, daughter of Byron, was born in 1815. Her mother, Annabella Milbanke, left Byron, who himself left England the following year — never to see his offspring again. In 1835 Ada married Lord King, who became the Earl and she the Countess of Lovelace in 1838.

Five years earlier, in 1833 (not long, incidentally, before she attempted to elope with her still unidentified tutor), Ada had first met Charles Babbage. They formed a lasting friendship in which she worked with and for him in the development of the first computers: the Difference Engine for computing tables and the general-purpose programmable Analytical Engine, which was never completed. It is for her association with Babbage that Ada is principally remembered, to the extent that she is said to have been the world's first computer programmer.

In her rich study of human drama and social history at the birth of the modern world, Dorothy Stein gives due accord to Ada's organizing abilities and her work in

translating and adding to Menabrea's monograph on the calculating engines. She does not, however, regard her as a major originator. The key historical questions revolve around the use of Jacquard punched cards to program the Analytical Engine. Dorothy Stein writes (p.93):

For the modern reader the important distinction . . . is that the Difference Engine followed an unvarying computational path (except for the parlour games Babbage played for the benefit of visitors such as Lady Byron), while the Analytical Engine was to be truly programmable and capable of changing its path according to the results of intermediate calculations or processes. Yet this was a feature mentioned by Ada only in passing . . . For Ada — as for Babbage — the calculating machine was a metaphor as well as a harbinger of economic and scientific progress. For example, the theme stressed in presenting the 'illustrations' or programs was that they demonstrate how certain lengthy, laborious, and complex calculations can be most efficiently executed . . . by the machine into recurrent cyclical groups. The same set of operations can then be repeated over and over by the engine, with only the starting and stopping points indicated by the instructions.

Ada, it seems, saw the programs, and her own hard-won knowledge and skills, in contemporary economic terms as *capital* to save labour and increase enterprise. But this interpretation hardly reduces her achievement: she clearly made a notable contribution to the working out of Babbage's ideas and to what at that time was (and perhaps still is) the metaphysics of computing, asking such questions as: "How can a machine handle imaginary

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The young Ada — "gifted with intense imagination".

Mary Evans

numbers?" It appears, though, that Babbage (born in 1792) was generally a couple of decades ahead of her in questions and answers, and infinitely ahead of everyone else. He himself remains a shadowy but dominating figure in Dorothy Stein's account.

Ada comes remarkably alive in this book, passionate but at times neurotic, with almost too much self-awareness, and falling into disasters ranging from illness and accident to losses on the horses — all this is wonderfully preserved in her correspondence and is most ably presented here. She was gifted with intense imagination. But she had but intermittent perseverance, even for bringing into being the calculating engines she had the wit to see were to be of such immense significance. □

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Glycans explored

Tim Hardingham

Glycoprotein and Proteoglycan Techniques. By J.G. Beeley. Elsevier: 1985. Pp. 462. Hbk Dfl. 295, \$109.25; pbk Dfl. 85, \$31.50.

CARBOHYDRATE structures can be one of the great turn-offs of student days. Be that as it may, the rise in interest in all aspects of the biological functions of glycoproteins and proteoglycans means that there is an ever-widening audience of people keen to get to grips with them. This book does much to help, for not only does it provide a compendium of useful laboratory techniques but also succeeds in conveying some of the essential flavour of research in this area; it gives a good, broad perspective on the variable and yet ordered structures shown by glycoproteins and proteoglycans, and a thorough chemical overview of what can and cannot be done to the intact glycoproteins and their isolated glycopeptides.

The early sections deal with largely tra-

ditional chemical methods of structural analysis. It is, however, the later parts, which review the use of specific enzymes and include sections on the battery of approaches possible with lectins and radioactive labelling techniques, that will be particularly useful. The range covered includes good examples of the variety of approaches that are possible for isolation, purification and structural analysis. Here, too, the author also addresses some of the problems frequently encountered when the supply of starting material is limited, as in characterizing products from cell culture.

The content is not beyond criticism — some of the newer approaches are not mentioned — but the emphasis on techniques accessible to any reasonably equipped laboratory means that the book will be ideal for those just starting up. Moreover it should enable both experienced and inexperienced readers to assimilate with much greater insight the wealth of current publications in this field.

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The ethics of human gene therapy

from LeRoy Walters

The correction of single-gene defects in humans by gene therapy is in sight, increasing the need for a solid framework for ethical evaluation.

WITHIN the next twelve months, it is likely that a proposal to perform gene therapy in humans will be submitted to local and national review bodies in the United States. The proposing research group will be based in one of the eight to ten laboratories in the world now pursuing molecular genetic approaches to the correction of selected inborn errors of metabolism. The hereditary disorders for which gene therapy will initially be attempted are quite rare—adenosine deaminase (ADA) deficiency, which produces severe combined immune deficiency, and Lesch-Nyhan syndrome, which leads to mental retardation and self-mutilation in children.

While gene therapy may sound revolutionary as an approach to human genetic disease, the techniques likely to be used in the near future closely resemble those of conventional medical practice. Thus, gene therapy for ADA deficiency can accurately be described as 'autologous bone marrow transplantation with an extra step'. That extra step will involve not the repair of a malfunctioning gene, but, rather, the addition of a properly functioning gene to as many target cells as possible, thereby compensating for the malfunction caused by the defective gene in those cells.

First steps

The early attempts at gene therapy will involve only somatic cells, especially the stem cells found in the bone marrow. Molecular genetic modification of germ cells in laboratory animals is still inefficient, and no research groups are currently contemplating the deliberate introduction of genetic alterations into the human germ line. Thus, fears about the transmission to future generations of intentionally induced genetic changes are misplaced, just as they would be after a kidney transplant. For the time being, moreover, only the simplest kinds of genetic defects are considered to be good candidates for gene therapy. These are single-gene defects which are recessively inherited and which result in the lack of an essential enzyme. The production of such enzymes is almost a mechanical function, necessary to be sure, but shared by humans with many other members of the

animal kingdom. Thus, any allegation that current gene therapy efforts represent 'tampering' with 'distinctively human characteristics' is simply fallacious.

Risk assessment

Ethical questions about gene therapy as now planned are of two kinds, substantive and procedural. The substantive questions can be further subdivided, with some oversimplification, into technical and non-technical questions. The chief technical questions surrounding gene therapy involve the comparison of potential benefits and harms, or, in contemporary language, risk assessment. The assessment process begins with an evaluation of the genetic disease to be treated. At this stage the central question is: What kinds of morbidity and what mortality rates are associated with the disease? If the disease is severe for the individuals affected by it, one can proceed to consider existing therapies and their effectiveness. If dietary or current medical therapies provide reasonable control for disease victims, the disease may not be a good early candidate for gene therapy. On the other hand, if no effective therapy exists or if the therapy cannot be used with some categories of patients, molecular genetic approaches to treatment may be a reasonable alternative to merely palliative measures.

Like the development of all innovative medical therapies, the pursuit of gene therapy as an alternative therapeutic mode involves preclinical studies in animal models and tissue cultures. At this stage the risk assessment process must seek to determine and evaluate the probable safety and effectiveness of the new technique. Here, researchers attempting to develop gene therapy techniques face a special, but by no means unique, problem: there are few laboratory animal models for the single-gene defects that afflict humans, and, in particular, there are no known non-human models for the enzyme-deficiency diseases which are likely to be judged appropriate early candidates for human gene therapy. Researchers may be able to insert a new gene into appropriate animal cells and monitor the expression of the gene, but they are unlikely to be able to demonstrate

a clinical 'cure' in a diseased laboratory animal.

From a safety standpoint, one advantage of a gene therapy approach that uses bone marrow cells is that the cells are treated outside the body, *in vitro*. If a particular experiment goes awry, the cells are simply not returned to the laboratory animal or, in the clinical context, to the patient. But other safety questions are matters of continuing concern. The new genes will probably be carried into the stem cells by carefully designed retroviruses from which much of the native genetic information has been deleted. These defective retroviruses will function as vectors for the new gene. It is possible, but not likely, that the retroviral vectors will recombine with undetected viruses or endogenous DNA sequences in the cells and so become infectious. It is also possible that the vector/gene combinations, because they integrate randomly into the chromosomes of the cells, will activate previously dormant proto-oncogenes or disrupt essential, properly functioning genes. On the question of efficiency, it is still not clear whether sufficiently high levels of expression can be achieved in primates to offer a reasonable hope of clinical benefit.

Other ethical issues

Non-technical questions about human gene therapy, while apparently less amenable to verification by laboratory data, are nonetheless important. These questions derive in part from the recent history of clinical innovation, from renal dialysis in the early 1960s, the first heart transplants in 1967 and recent experience with the artificial heart and xenografting. They are also based on codes of ethics in research published between the late 1940s and the present.

In the early years of renal dialysis, one major issue was the selection of a few patients from among many candidates for life-saving treatment. In Seattle, Washington, a special committee was established to make these decisions. Commentators on the Seattle committee's experience have noted how difficult it is to avoid making judgements about the comparative social worth of patients when not all patients can be offered treatment¹. With

rare genetic diseases, although the potential patient pool will be small, choices will need to be made from among multiple candidates for experimental gene therapy. Thus, fairness in the selection of subjects may be an important issue even in the earliest trials of human gene therapy.

Informed consent will almost certainly also be an important issue. To be adequately informed about their decisions, prospective patients will need to have, or have imparted to them, basic information about bone marrow transplantation and gene therapy techniques. If, as seems likely, parents or guardians are asked to make decisions on behalf of infants or young children, the consent process will be even more complex. Properly informed, potential research subjects or their proxies will be made aware that they are embarking on essentially uncharted territory. This awareness should help to temper their hope—and that of the investigators, also—that they are participating in a major therapeutic breakthrough.

Questions of privacy and confidentiality may also arise. The pioneer recipients of heart transplants and the artificial heart are known to us by name; in contrast, the newborn recipient of a baboon heart, Baby Fae, remained at least partially anonymous until her death in late 1984. With gene therapy, one hopes for a reasonable balance between familial privacy and the desire of the public and the media to know. Researchers will bear the primary responsibility for informing patients and their families of the public interest in gene therapy and for shielding patients from excessive media exposure. If, as seems likely, the earliest patients are infants or young children who are incapable of expressing their own views regarding publicity, they will deserve special protection.

Guidelines

In the Declaration of Helsinki and similar codes of ethics in research, biomedical researchers have proposed general standards for human experimentation. The revised Declaration of Helsinki², adopted by the World Medical Association in 1975, includes ethical guidelines on research design, risk-benefit analysis, informed consent, privacy and accuracy in reporting research results. In most developed nations, local committees, variously called 'ethics committees' or 'institutional review boards', rely on these international standards and derivative national rules in reviewing individual research protocols. However, when major therapeutic innovations are initially proposed, this carefully evolved approach to the review of proposed human experimentation may need to be supplemented by more specific ethical standards and, possibly, by custom-tailored review mechanisms. *In vitro* fertilization is an example of a new biomedical technology that has been the

object of special scrutiny in several countries, including the United Kingdom, Australia and Canada.

During the past two-and-a-half years, a framework for the ethical evaluation of human gene therapy has been developed in the United States. This framework includes a publicly discussed set of questions, called *Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols*³, and a public national review process. The *Points to Consider* reflect a national and perhaps international consensus on the most important areas of concern surrounding human gene therapy. Major areas identified in the central section of the document are the following:

- (1) Objectives and rationale of the proposed research.
- (2) Research design, anticipated risks and benefits: (i) structure and characteristics of the biological system; (ii) preclinical studies; (iii) clinical procedures, including patient monitoring; (iv) public health considerations; (v) qualifications of investigators and adequacy of facilities.
- (3) Selection of patients.
- (4) Informed consent.
- (5) Privacy and confidentiality.

A series of questions is posed about each area of concern, and great latitude is left to investigators in formulating responses to the questions. For example, on the subject of preclinical studies with laboratory animals, investigators are asked: "Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results?"

The *Points to Consider* document was drafted by the Working Group on Human Gene Therapy, an interdisciplinary subcommittee of the NIH Recombinant DNA Advisory Committee (RAC). The Working Group includes three laboratory scientists, three clinicians, three ethicists, three attorneys, two public-policy specialists, and a lay member. The document represents an attempt to distill 15 years of ethical discussion in published articles and books⁴⁻¹⁵, at public symposia¹⁶⁻¹⁸, and in government hearings and reports in the United States and Europe¹⁹⁻²². *Points to Consider* was published twice for public comment in the United States *Federal Register* and was revised to take into account the suggestions made by respondents.

As a complement to the confidential review of new biological compounds conducted by the federal Food and Drug Administration, a special public review mechanism has also been established to evaluate proposals to perform gene therapy in humans, at least during the early years. This review mechanism was created by the NIH RAC and is modelled after the review process employed by RAC during the early years of recombinant DNA research. After review by local ethics

committees, RAC and its subcommittee, the Working Group on Human Gene Therapy, will provide public national review of each gene therapy proposal that is to be supported by NIH funds. All interested persons will therefore have ready access to information about gene therapy proposals, to the review process itself and to its outcome.

When gene therapy has not yet cured a human patient of disease, it may seem premature to discuss questions that may emerge if human gene therapy is successful. Yet, one role of ethics is to provide timely consideration of future questions for public policy. Several issues may arise.

Genetic diagnosis

At present, attempts to describe 'the morbid anatomy of the human genome' are outpacing gene therapy by a considerable margin²³. Within the past year, new markers for several major genetic diseases have been described, among them cystic fibrosis²⁴⁻²⁶, polycystic kidney disease²⁷ and Duchenne muscular dystrophy²⁸. At the prenatal stage, early diagnosis of genetic defects presents couples with a decision about selective abortion, but applied to children or adults, the new tests will provide early notice of a tendency to develop a particular disease (such as atherosclerosis²⁹) or of the presence of a gene that will cause a late-onset disorder (such as Huntington's disease³⁰).

The 'early notice' aspect of the new genetic tests will raise difficult ethical questions in its own right. For example, which children or adults should be tested, and what should they be told about the results of the tests? Moreover, one can anticipate that insurance companies, employers and perhaps even prospective marriage partners, will have a keen interest in securing detailed genetic profiles of particular individuals. The primary implication of these screening techniques for gene therapy is that they may identify additional groups of candidates for curative or pre-emptive intervention.

Commercial considerations

Commercially oriented biotechnology companies have been leading participants in the quest for DNA-based diagnostic techniques but seem, at present, relatively uninterested in DNA-mediated approaches to the cure of disease. This lack of interest may reflect the fact that, in the near future at least, human gene therapy will be a labour-intensive procedure, akin to bone marrow transplantation. Each university-based research team involved with gene therapy also seems to be tailoring its own vector/gene combinations to suit its specific purposes. It is possible that there may emerge commercial interest in producing vectors or vector/gene kits for particular diseases, but, given the rarity of the diseases thought to be the likeliest candi-

dates for early attempts at gene therapy, commercial incentives are currently quite weak.

Gene replacement

Present approaches to gene therapy can quite literally be described as gene addition. Properly functioning genes, introduced into the appropriate cells, will, it is hoped, compensate for malfunctioning or nonfunctioning genes. This approach holds great promise for the somatic-cell treatment of recessive genetic disorders, but for the treatment of dominant disorders such as Huntington's disease, it may be necessary to inactivate the disease-causing gene or even to remove and replace it with a gene that functions properly. Techniques for this kind of precisely targeted molecular microsurgery have not yet been developed for mammals and will probably not emerge for decades.

Germline therapy

No aspect of gene therapy is more highly charged than that of germline or germ-cell therapy; it might seem, therefore, politically prudent to avoid the subject. But what is politically prudent may not be ethically responsible. In fact, timely ethical discussion of this issue, before germline gene therapy in humans is technically feasible, may assist future policymakers in their deliberations.

The principal rationale for germline human gene therapy, when it becomes a technical possibility, will be a simple argument from efficiency. If somatic-cell therapy becomes a successful cure for single-gene defects of high prevalence, such as cystic fibrosis and sickle-cell anaemia, phenotypically normal patients will grow to adulthood and presumably be capable of reproducing. They will then constitute a new group of homozygous 'carriers' of genetic disease, who can transmit malfunctioning genes to their offspring. Affected offspring could presumably be treated by means of somatic-cell gene therapy in each succeeding generation, but some phenotypically cured patients would probably consider it more efficient to prevent the transmission of specific malfunctioning genes to their offspring, if the option were available.

A second rationale for the germline approach is that some genetic diseases may be treatable only by this method. For example, because of the blood-brain barrier, the brain cells involved in hereditary central nervous system disorders may be inaccessible to somatic-cell gene therapy. Early intervention that affects all the cells

of the future organism, including the germ cells, may be the only means available for treating cells or tissues which are not amenable to genetic repair after birth.

Genetic changes have been introduced into the germ lines of several species of laboratory and domestic animals³¹⁻³⁴, but the method for producing such transgenic animals is unlikely to be used with humans. Because the foreign DNA is inserted into the pronuclei of mammalian embryos before the pronuclei fuse and the new genome is established, one would not know in most cases whether a particular embryo were destined to have a genetic disease. A more likely approach in humans is the genetic repair of sperm or egg cells, either *in vivo* or, more probably, *in vitro*. Methods for reliably introducing properly functioning genes into germ cells and for verifying their successful introduction would need to be developed. As simple gene addition would allow the transmission of known malfunctioning genes to future generations, the techniques of gene inactivation or gene replacement would probably be used, if available.

Economic issues

Given that the first-year costs of cardiac transplantation approximate \$90,000 per patient (1983 dollars) in the United States³⁵, it may reasonably be asked whether gene therapy will be another expensive half-way technology. If employed with bone marrow transplantation, human gene therapy will be labour-intensive and will entail sizeable initial costs. But even if one does not assign an economic value to improvement in the quality of cured patients' lives, the one-time cost for gene therapy may be considerably less than the cost of repeated hospitalization for the treatment of sickle-cell anaemia or cystic fibrosis. In short, for patients suffering from many, if not most, genetic diseases caused by single-gene defects, gene therapy may become a cost-effective method of care.

In the more distant future, the prospects for, and possible approaches to, human gene therapy are not yet clear. But if the link between gene therapy and bone marrow transplantation can be broken, gene therapy may become available to a wider circle of patients. For example, if vectors specific for particular target cells can be developed, vector/gene combinations can perhaps be administered intravenously to outpatients. At that stage, if it is ever reached, medicine will stand at the threshold of a new era in treating the more than 3,000 genetic disorders that afflict our species.

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Transitional field behaviour from Southern Hemisphere lavas: evidence for two-stage reversals of the geodynamo

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Palaeomagnetic records from Australasian basalts provide a rare view of transitional field behaviour, as seen from mid-southern latitudes. These data suggest that a geomagnetic reversal is accomplished in two steps, separated by an interval of relative dynamo quiescence. A potentially long-lived field configuration, developed early, is observed to be the 'stepping stone' from which both successful and unsuccessful reversal attempts are made. Several aborted attempts involving the same intermediate field geometry may precede an actual polarity transition.

WHILE the number of palaeomagnetic records of polarity transitions obtained from sites in the Northern Hemisphere has steadily increased¹⁻⁸, the need for Southern Hemisphere records has become more apparent. Although one of the first detailed accounts of palaeofield behaviour during a complete reversal comes from South Africa⁹, few additional records from sites south of the Equator have since been reported^{10,11}. Nevertheless, it is clear that any meaningful determination of field morphology during transitions, a major constraint on any complete model of the reversing geodynamo, requires contemporaneous records of intermediate field behaviour from sites scattered about both hemispheres¹²⁻¹⁴.

To help rectify this situation, three mid-southern-latitude Cenozoic shield volcanos which were the focus of magnetostratigraphic studies some fifteen years ago^{15,16} were revisited (Fig. 1a). Specifically, the sampled sites are:

- Liverpool Volcano (31.7°S, 150.2°E), New South Wales, Australia, where a continuous Oligocene sequence of approximately 59 flows records a series of 4 geomagnetic events, L1 to L4.

- Akaroa Volcano (43.8°S, 173.0°E), Banks Peninsula, New Zealand, where a Miocene lava sequence records intermediate field behaviour through two sequential reversals, A1 and A2.

- Mt Kaputar (30.2°S, 150.2°E), New South Wales, Australia, where a late Miocene excursion (K1) is recorded.

The data from these palaeomagnetic events have been corrected for any subsequent tectonic rotation of the sites and have been reduced to site palaeolatitudes consistent with the age of the lavas. Both corrections are deduced from the averaged full-polarity data for each site. The volcanic structures appear to be flat-lying, so that correction for tectonic tilt was unnecessary.

Recorded field behaviour

Liverpool Volcano, Australia. This volcano, potassium-argon dated at 33.7 ± 0.7 Myr (ref. 15), is built of alkali olivine basalts. Previously reported palaeomagnetic data from several lava flows have been interpreted as showing a series of three polarity reversals¹⁵; however, we find that only one complete transition, involving a polarity change from reverse-to-normal (R-N), is recorded at Liverpool. We believe that this discrepancy is caused by a small number of palaeomagnetic directions recorded by an unusual magnetic carrier mineral¹⁷.

Approximately 300 cores were drilled over ~360 m of continuous vertical section exposed along Yarraman Creek and two of its tributaries. A dense and sometimes redundant sampling procedure was used to ensure that the magnetic record had no gaps. Each site was examined carefully to verify that the flows

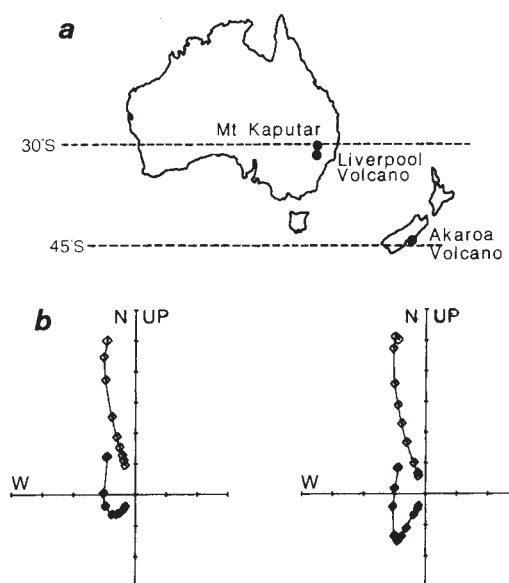


Fig. 1 a, Location of Cenozoic volcanos sampled for this study. b, Orthogonal plots of remanence behaviour during stepwise alternating field (AF) demagnetization to 20 mT (left) and stepwise thermal demagnetization to 320 °C (right) conducted on typical specimens from flow YC21 from Liverpool Volcano. This flow was chosen because it possesses the most intermediate primary palaeofield direction of all flows reported in this study. Solid (open) symbols denote projections on the vertical (horizontal) plane.

were definitely *in situ*. In all, 59 individual flows and flow groups were sampled in detail.

Palaeomagnetic analysis involved both alternating field (AF) demagnetization and thermal cleaning, sometimes in combination. At no time was single-step or 'blanket' demagnetization employed. For each specimen, the direction of primary thermoremanent magnetization (TRM) was determined from the observed behaviour during stepwise demagnetization (see Fig. 1b).

In Fig. 2a, declination (D) and inclination (I) are plotted in chronological flow order; the flows in this sequence provide a record of field behaviour spanning a single R-N transition.

Inspection of Fig. 2a reveals that nearly the entire reversal appears to take place between two successive extrusions, but that a large number of flows in the sequence, before and after the primary transition, display intermediate palaeodirections. Lack of information regarding the absolute timing of lava extrusion means that we cannot tell what time-span is involved.

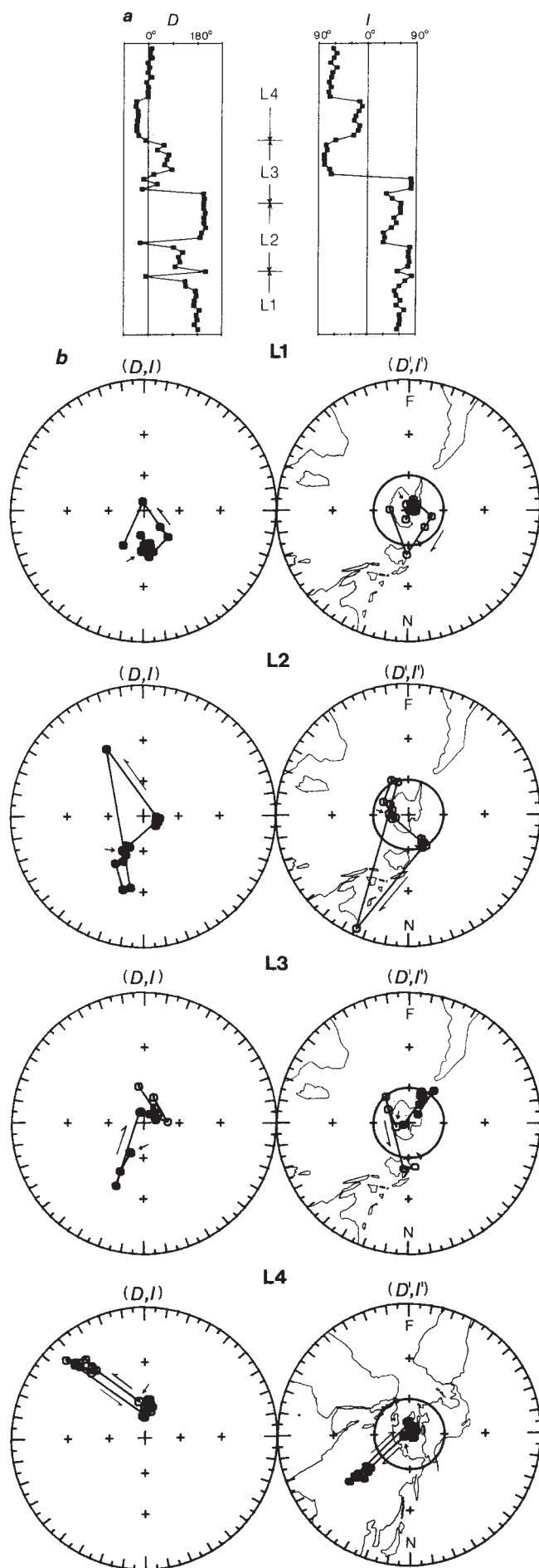


Fig. 2 AF- and thermally demagnetized mean flow directions from a continuous sequence of basalts from Liverpool Volcano, Australia. *a*, Declination (*D*) and inclination (*I*) from transitional events L1 (R-R; the oldest), L2 (R-R), L3 (R-N) and L4 (N-N). *b*, Polar stereographic plots in (*D*, *I*)-space (left) and in rotated (*D'*, *I'*)-space¹⁸ (right). Solid (open) symbols correspond to palaeodirections on the lower (upper) hemisphere. The four events are displayed chronologically. (*D'*, *I'*) plots present palaeodirectional movement as if one were looking down the normal polarity dipole field direction; the angular distance to any point from the centre is a direct measure of the angular distance from the axial dipole field direction corresponding to this site in Oligocene times. The small circle represents a 30° departure and the small plus signs represent a 60° departure. 'N' ('F') indicates perfectly near-sided (far-sided) rotated directions. The double-headed arrow indicates the oldest datum of each sequence. Broken lines are used to indicate unknown directional paths of large angular extent. Those (*D'*, *I'*) directions corresponding to virtual geomagnetic poles (VGPs) lying on the coastlines of the continents are indicated. In this way one can observe the VGP path in this directional space.

In Fig. 2*b*, the Liverpool data are displayed as vector paths in (*D*, *I*)-space and rotated (*D'*, *I'*)-space¹⁸. Before the actual change in polarity, three departures of the field vector from full polarity are seen (Fig. 2*b*, events L1-L3). The first directional swing (L1) involves a maximum recorded angular displacement from the axial dipole field direction of ~35° in the north/south-up/down plane, to subvertical (downwards), and is an almost perfectly near-sided^{12,13} direction. The second swing (L2), also strongly near-sided, has a maximum recorded extent of about 90° from full polarity. Note that this single-flow result is preceded by a direction similar to that associated with the first swing and, moreover, that this recurring, strongly near-sided palaeofield orientation remained virtually stationary during the extrusion of several flows.

Interestingly, the third directional swing (L3) again proceeds to a strongly near-sided intermediate direction, very similar to the other such directions noted. This time, three successive flows record this palaeofield orientation. Immediately following these intermediate directions is the (possibly rapid) transition to normal polarity. After a final directional swing (L4), marginally near-sided, to a position ~45-60° from the direction associated with the (now normal-polarity) axial dipole field, the palaeofield appears to 'settle' into a long-term normal polarity state.

Akaroa Volcano, New Zealand. The Miocene sequence of alkaline lavas at Akaroa Volcano, K-Ar dated at ~8.5 Myr, contains a number of polarity boundaries¹⁶. In our resampling, approximately 250 cores were drilled over ~160 m of section exposed along Lighthouse Road. Figure 3 shows the data from two sequential transitions (a N-R transition followed by a R-N transition).

Although the record from this shield volcano appears to be less detailed than that from the Liverpool sequence, both transitions do contain intermediate palaeofield directions. Specifically, each reversal record contains a single, localized set of intermediate directions; in the case of the R-N reversal (Fig. 3, event A2), these directions remained nearly invariant during the extrusion of seven lavas. As at Liverpool Volcano, the R-N transition recorded at Akaroa Volcano includes intermediate directions which deviate by up to 45° in the north/south, up/down plane from the reverse-polarity axial dipole field direction; however, in contrast to the Liverpool record, this intermediate orientation is almost perfectly far-sided. That is, while the Liverpool field behaviour during events L1-L3 is dominated by a steepening in inclination, that recorded at Akaroa during event A2 exhibits a shallowing. The intermediate field directions recorded during the apparent onset of the preceding N-R transition (Fig. 3, event A1) also deviate from the axial dipole field direction by about 45°; however, for this event the orientation of the directional swing is far from the north/south-up/down plane.

Between the recorded N-R and R-N transitions, the Akaroa lavas may have recorded as many as three R-R palaeofield

excursions. Unfortunately, two of these possible events are each observed in only one lava. The remaining event is recorded in five lavas; however, the magnetic remanence properties of the three oldest flows of this sequence are atypical and require further examination. The two youngest flows, which exhibit no unusual remanence properties, possess palaeofield directions which are very close to the intermediate directions seen in the subsequent R-N transition. Such an occurrence is reminiscent of the Liverpool data.

Mount Kaputar, Australia. Previously reported palaeomagnetic results from the Mt Kaputar lava sequence, K-Ar dated at ~ 17.5 Myr, suggested the presence of a single, normal-polarity geomagnetic excursion¹⁵. Our sampling confirmed this event; Fig. 4 shows the excursion to be almost perfectly far-sided, involving a directional change from full polarity to sub-vertical (upwards). Moreover, as at both Liverpool and Akaroa, this event consists of a single characteristic direction recorded in several successive lavas. It is not known whether event K1 is ultimately followed by a full polarity transition.

Recurring intermediate directions

Whether associated with a geomagnetic excursion or with a polarity transition, the intermediate behaviour of the palaeomagnetic field revealed in the above mid-southern-latitude records appears to be dominated by an angular displacement to a position between 30° and 60° from the initial axial dipole field direction. Figure 5 summarizes the intermediate directional data reported here, plotted in (D', I') -space. The almost complete lack of intermediate directions deviating by more than 60° from the initial axial dipole direction is striking; indeed, only one of the 31 flows possesses such a direction.

The similarity in directional behaviour recorded during these events raises the possibility that most of them, perhaps all, have their origin in the same type of dynamo process in the Earth's core. The data suggest that the geomagnetic reversal process is characterized at its onset by an essentially stationary intermediate field geometry which, at least at mid-southern-latitude sites, is associated with vector directions less than $\sim 60^\circ$ from that of the immediately preceding axial dipole field. In particular, the palaeofield behaviour recorded in the Liverpool lavas appears to possess a 'memory', insofar as each of a series of three departures is apparently dominated by essentially the same intermediate field geometry (Fig. 2b, events L1-L3). This observation of a recurring transitional direction strongly suggests a temporal dominance of the reversing field by a stationary or quasi-stationary transitional configuration. This interpretation of the Liverpool data does not rely on any assumptions regarding the mean extrusion rate or degree of eruptive clustering.

These data demonstrate a clear observational link between successful polarity transitions and the occurrence of at least some geomagnetic excursions (abortive field reversals). Moreover, the Liverpool results indicate that the initial core process associated with field reversals is the same regardless of whether a given attempt is successful or unsuccessful.

Another instance of a recurring intermediate field geometry (this time probably not associated with an ultimately successful polarity transition) can be found in the literature. Reporting on a palaeomagnetic investigation of Brunhes-age lavas from Amsterdam Island, situated at mid-southern latitudes (37.8° S, 77.5° E), Watkins and Nougier¹⁹ note that two distinct basalt sequences (containing two and three flows, respectively) display nearly the same intermediate palaeodirection. They then argue that the geomagnetic field vector recorded at Amsterdam Island changed from full normal polarity to the same intermediate direction on two separate occasions. Interestingly, these intermediate Brunhes directions are found to lie between 30° and 45° from the initial (normal polarity) axial dipole field direction and, moreover, are strongly far-sided. In fact, this intermediate behaviour corresponds to a near-vertical (upwards) vector field

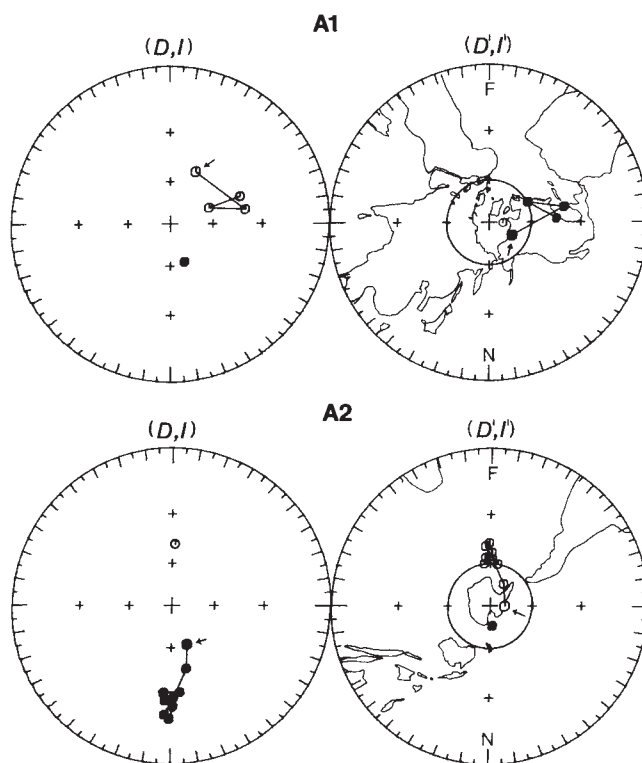


Fig. 3 Demagnetized directional data from two transitional events from Akaroa Volcano, New Zealand: A1 (N-R; the older) and A2 (R-N). See Fig. 2b legend.

direction at the site, strikingly similar to that found in the (normal) Miocene lavas at Mount Kaputar and nearly antipodal to the (reverse) Oligocene lavas from Liverpool Volcano. The Amsterdam Island events are included in Fig. 5 (Am1, 2).

These younger Southern Hemisphere data, taken together with those from Australia and New Zealand, suggest that the core process responsible for these intermediate-field events has possessed similar characteristics throughout the Cenozoic. Moreover, when considered in this light, the Amsterdam data indicate that at least some unsuccessful reversal attempts have occurred during the Brunhes normal epoch. It seems likely that such attempts have characterized the behaviour of the geodynamo throughout geological time.

With regard to field geometry, Fig. 5 indicates strikingly near-sided or far-sided intermediate directions to be present during all four recorded events involving reverse-polarity fields, as well as three of the five events involving normal polarity. Of these seven departures from full polarity consistent with a strongly axisymmetric, apparently stationary intermediate field geometry (namely, L1-3, A2, K1 and Am1, 2), six are associated with an inclination steepening, while event A2 alone indicates an inclination shallowing. The two remaining normal polarity departures (namely, L4 and A1) are associated with significant non-zonal field characteristics. Hence, although many of these data suggest largely axisymmetric fields of varying harmonic content during the onset of reversals, while the field is transitional and essentially stationary, such a geometrical aspect may not always be associated with the core process.

The lack of intermediate directions deviating by more than 60° from the initial axial dipole field direction during, in particular, the three successful transitions suggests that field changes are rapid during the hypothesized second stage of the reversal process. The first (onset) stage may also be rapid, but in view of the lack of knowledge regarding the recurrence time between any two extrusions, these suggestions must be considered statistically. In this sense, however, the summary display of the Southern Hemisphere basalt data (Fig. 5) is quite compelling.

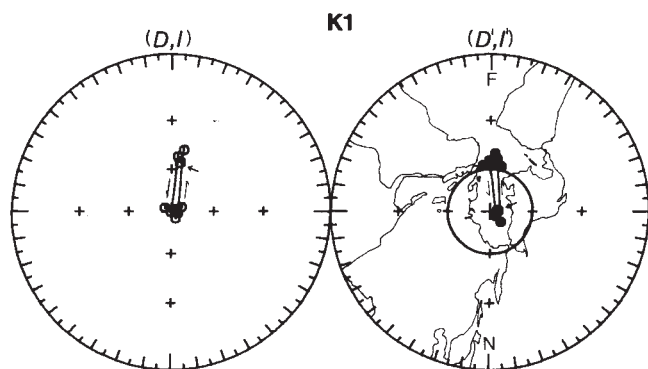


Fig. 4 Demagnetized directional data from event K1 (N-N) from Mt Kaputar, Australia. See Fig. 2b legend.

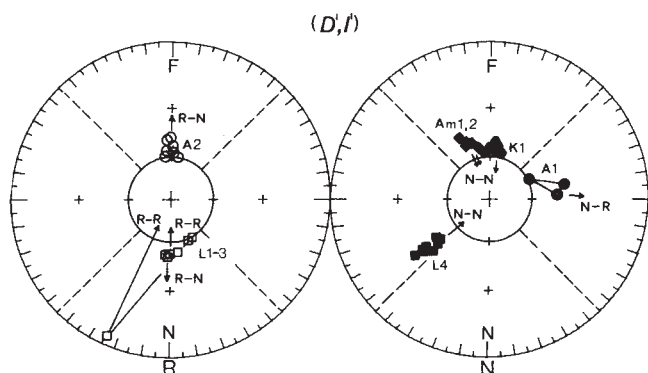


Fig. 5 Summary plot in (D', I') -space of all intermediate directions reported in this study. Data corresponding to two Brunhes-aged excursions (Am1, 2) from Amsterdam Island, Indian Ocean¹⁹ are also plotted. Events starting from a reverse (normal) polarity field appear at left (right). The diagonal dashed lines are the boundaries between directional quadrants consistent and inconsistent with axisymmetric field dominance¹⁸. See Fig. 2b legend.

Comparison with Northern Hemisphere records

The question that arises is: to what degree are palaeomagnetic transition records from the Northern Hemisphere compatible with these Southern Hemisphere data in suggesting the existence of a two-step reversal process?

The literature contains several apparently highly reliable Cenozoic records from igneous structures consistent with our Southern Hemisphere findings. A dramatic record of this kind is the Icelandic 'R3-N3' transition record²⁰, which consists almost exclusively of a large number of systematically varying, intermediate palaeodirections residing less than 90° from the reverse-polarity axial dipole field direction¹⁸. Reporting the measurement of strong palaeointensities in a few of these intermediate-directed lavas, Shaw^{20,21} proposed that a third metastable state of the geomagnetic field, in addition to normal and reverse polarity, may exist. We return to this hypothesis later.

More recently, Bogue and Coe^{3,22} have investigated two sequential reversals obtained from lava sequences on Kauai, Hawaii. They report the existence of clustered, marginally intermediate directions at the onset of the reversals (especially for the R-N transition), but find a complete lack of strongly intermediate directions at other times during the polarity transitions. From these data, Bogue and Coe argue that the bulk of the directional changes associated with both transitions occurred rapidly.

The recently reported reinvestigation of the very detailed Miocene R-N record from the mid-northern-latitude basalts comprising Steens Mountain, Oregon⁸, reveals that the core process is characterized by stop-and-go behaviour. The authors⁸

consider such behaviour to be a consequence of a succession of 'geomagnetic impulses' in the core. In particular, the authors note a large and regular change in direction at the onset of the reversal to an intermediate orientation which remains essentially unchanged during the extrusion of several flows. There follows an apparently rapid change (an impulse) to full normal polarity and then, before the completion of the reversal, a 'rebound' to the same intermediate field direction⁸.

Stronger supporting evidence comes from intrusions, because of their capability to record in a more continuous fashion than lava sequences. In particular, the detailed records of two Miocene R-N transitions obtained from intrusions in the Pacific north-west of the United States²³, which differ in age by several Myr, display strikingly similar palaeofield behaviour^{1,23}. Specifically, both records indicate a quite rapid and marked change in inclination at the onset of the transition. The field is then observed to maintain a loosely stationary intermediate geometry, apparently for quite some time before the completion of each reversal. One record (Tatoosh intrusion) contains a rebound from normal polarity to this same field configuration²³, much as is seen in the Steens Mountain record. Given that these records were derived from slowly cooled igneous bodies and are thus considered to be continuous, such time-dependent interpretations of directional movements are highly credible.

Particular high-resolution reversal records obtained from sedimentary structures in the Northern Hemisphere also display field behaviour consistent with our findings from southern latitudes. The two most striking examples are the Matuyama-Brunhes record from the Boso Peninsula, Japan²⁴, and the Gauss-Matuyama record from Searles Valley, California⁵. Both records suggest the reversal onset to be characterized by a movement to an intermediate palaeodirection which remains essentially stationary. In the younger (R-N) transition, the intermediate field configuration displays strong zonal symmetry¹; however, the intermediate field recorded during the older (N-R) transition is without question non-zonal.

The geomagnetic reversal process

Clearly, a significant fraction of available transition data from both hemispheres supports the idea of a reversal process in the core which is rather discontinuous. We believe that the findings from Southern Hemisphere volcanics presented here further clarify the core mechanism. Specifically, these data suggest that a potentially long-lived, intermediate field state is developed near the onset of the transition process. From this stationary or quasi-stationary configuration, a rapid change to either polarity is indicated.

It seems reasonable to suspect that geomagnetic reversal attempts start locally in the core²⁵, perhaps caused by a form of short-range, hydrodynamic disturbance. Moreover, the repetitive field behaviour recorded at Liverpool Volcano may indicate that a succession of nearly identical disturbances can occur at the same location in the core over a relatively short interval of time, or alternatively, that a single disturbance can fluctuate significantly in intensity during its existence. The developed intermediate field geometry associated with a given attempt may be considered metastable as first proposed by Shaw^{20,21}. However, according to the Southern Hemisphere data, the particular geometry is likely to vary from one series of events to the next, perhaps linked to the location in the core where the reversal-causing disturbance initiates. At this point in the process the reversal attempt may abort. It is not known just what conditions are required for the dynamo to undergo a successful transition in polarity.

As we have seen, available high-resolution transition data often suggest quite rapid directional movement to be a feature of the reversing geodynamo. Indeed, one conclusion from the Steens Mountain study⁸ is that a small number of geomagnetic impulses, characterized by extremely large angular rates of change of the vector field, occurred during this reversal. Such

rapidity indicates that reversal attempts are in large part 'fluid-driven'; that is, associated with changes dominated by frozen-in flux^{26,27} in a changing velocity field. On the other hand, the process of magnetic flux diffusion must become significant over longer intervals of time. This may explain the observation, certainly a feature of much of the Southern Hemisphere data, of apparent axisymmetry of the stationary or quasi-stationary transitional field geometry developed between the two stages of more rapid change. Specifically, as the duration of a reversal-causing hydrodynamic disturbance increases, so too may the zonal symmetry of the perturbed field produced by the affected local fluid source. In an $\alpha\omega$ -dynamo^{28,29} this may result from flux diffusion as the ω -process spreads the altered field azimuthally. The data, however, cannot eliminate the possibility that those cases of apparent zonal dominance are produced from the outset by latitudinal 'flooding'^{12,13,22} or by diffusive decay of an axially symmetric field³⁰. Neither can the recorded events, each having been obtained from a single site, provide any quantitative measure of the relative importance of zonal and non-zonal harmonic content.

We emphasize that no compelling information is gained from the Southern Hemisphere data regarding the nature of configurational changes throughout the reversal process. Hence, any suggestion of axisymmetry associated with a given event cannot be extrapolated, especially to times after the development of the apparently stationary intermediate field configuration. Indeed, the data suggest the return to full polarity during successful attempts to be accomplished by one, or a series of⁸, rapid, fluid-driven events. Such are not

likely to be associated with axially symmetric fields²⁷.

The Southern Hemisphere transition data clearly suggest that a stationary or quasi-stationary intermediate field configuration may dominate an entire reversal. This interpretation of the data, if valid for most polarity reversal attempts, implies that the corresponding global field geometry associated with a given event is quantifiable, provided that the same feature is recognized in contemporaneous, distant records. When the appropriate data become available, such a determination will constitute a major step forward in our understanding of the spatial characteristics of the reversal mechanism in the Earth's core.

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Transfer of specificity by murine α and β T-cell receptor genes

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T-cell receptor α - and β -chain genes were isolated from a class I major histocompatibility complex-restricted cytotoxic T-cell clone and transferred by protoplast fusion into another cytolytic T-cell clone of different specificity. Expression of the transfected α and β genes endowed the recipient cell with the specificity of the donor cell.

IN the immune system of vertebrates, T cells recognize foreign structures on cell surfaces in the context of glycoproteins encoded by the major histocompatibility complex (MHC) (for review see ref. 1). The molecule that recognizes the foreign antigens and MHC determinants—the T-cell receptor—is composed of at least two polypeptide chains, α and β , which

contribute to the specificity of the cell (see refs 2, 3 for a review). The α - and β -chains are associated on the cell surface with additional molecules, notably the T3 molecule, which might be important for certain effector functions^{4,5}. Furthermore, the L γ 2/T8 and L3T4/T4 surface glycoproteins are thought to stabilize the binding of T cells to their targets⁶⁻⁸. It is assumed that these

additional interactions are especially important for receptors with low affinity as the blocking effect of antibodies directed against L3T4 can be reversed by increasing the density of the target antigen^{7,8}.

The genes encoding the α - and β -chains of the T-cell receptor have been cloned and characterized⁹⁻¹¹; they are composed of genetic segments which rearrange during T-cell development to generate T cells with clonally distributed receptors containing variable (V) and constant (C) regions. In the mouse, the variable regions of the α - and β -chains are encoded by about 20 V_β , 2 D_β (diversity), 12 J_β (joining), at least 50 V_α , and over 20 J_α gene segments, while a single C_α and two almost identical C_β genes encode the constant regions¹²⁻²³. A third gene family, γ , is closely related to the α and β genes and shows specific rearrangements in T cells²⁴⁻²⁶. The protein product of the γ gene has not yet been identified and the function of the γ gene is unknown.

Various elegant experiments have addressed the question of whether the α - and β -chains are sufficient to endow a T cell with both antigen and MHC specificity^{27,28}. Perhaps the most straightforward way to investigate this question is by gene transfer between T cells of different specificities. Here we show that T-cell receptor α - and β -chain genes, isolated from a cytotoxic T cell, are sufficient to transfer that cell's MHC-restricted antigen specificity to another T cell.

Isolation of α - and β -chain alleles

The cytotoxic T-cell clone BDFL 1.1.3 (called BDFL), isolated from a (C57BL/6 $\times$ DBA/2) F_1 mouse shows specificity for the hapten fluorescein (FL) and the MHC class I antigen D^b. We constructed complementary DNA and genomic DNA libraries for BDFL using λ gt11 and cosmid vectors, respectively. From the cDNA library we isolated two α -chain and four β -chain cDNA clones using α - and β -chain constant-region hybridization probes that have been described elsewhere^{29,30}. Sequence determination of the cDNA clones revealed that the two α and all four β clones were derived from a single, presumably functional, α - and β -chain allele, respectively. Northern blot analysis of BDFL poly(A)⁺ RNA with α - and β -chain constant-region probes revealed 1.5-kilobase (kb) and (less abundant) 1.2-kb α -chain transcripts and a 1.3-kb β -chain transcript (Fig. 1). The α -chain cDNA clones correspond to the larger transcript, as shown using the V-region-specific hybridization probe $V_{BDFL\alpha I}$ (Fig. 1).

Cosmid clones were isolated from the genomic library²⁹ using α - and β -chain constant-region probes as well as the $V_{BDFL\alpha I}$ variable-region probe. The $V_{BDFL\alpha I}$ probe detects only a single V gene segment in germline DNA (data not shown). Based on overlapping restriction maps, these clones could be ordered into four clusters defining the two α - and the two β -chain alleles of BDFL (Fig. 2). Comparison of the cloned genes with germline DNA sequences for α - and β -chain genes^{14,22} revealed that all four alleles had undergone DNA rearrangements.

Identification of functional alleles

To characterize the four alleles, we determined their DNA sequences around the points of rearrangement. The αI and βI alleles are functionally rearranged genes while the αII and βII alleles contain nonfunctional rearrangements. All four genes possess rearranged V gene segments (Fig. 3).

The two α alleles have been formed by rearrangement of two V gene segments, termed $V_{BDFL\alpha I}$ and $V_{BDFL\alpha II}$, with J_α gene segments located 40 and 10 kb upstream of C_α , respectively (Figs 2a, b and 3). The two V_α and the $J_{BDFL\alpha I}$ gene segments have not been described previously, whereas the $J_{BDFL\alpha II}$ gene segment has been found²⁰ in an α -chain cDNA clone. We also sequenced the $J_{BDFL\alpha II}$ gene segment in a germline clone derived from BALB/c DNA (Figs 2a, 3).

The BDFL αI allele contains an open reading frame throughout the V gene and a splice site at the expected position

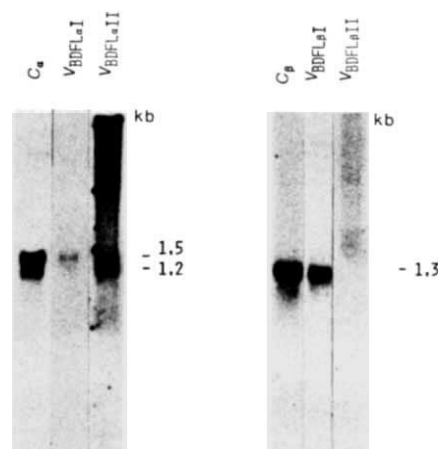


Fig. 1 Northern blot analysis of T-cell receptor genes of the cytotoxic T-cell clone BDFL. Poly(A)⁺-selected RNA (2 μ g per lane) was separated on a 1% formaldehyde gel, transferred to a nitrocellulose filter and hybridized with the probes indicated. The C_α probe is identical to probe 2 described previously²⁹. The $V_{BDFL\alpha I}$ probe is an *RsaI*-*EcoRI* fragment of the α -chain cDNA clone pTBD1.9, isolated from the BDFL cDNA library. This probe contains 200 bp of the BDFL αI V region and 25 bp of the C_α region. The $V_{BDFL\alpha II}$ probe is a 4-kb *BamHI* fragment containing the V- $J_{BDFL\alpha II}$ exon from cosmid clone BDFL9.3 (compare with Fig. 2). The C_β probe is the 300-bp insert of the β -chain cDNA clone 4.4 described previously³⁰. The $V_{BDFL\beta I}$ probe is a 200-bp *EcoRI*-*PstI* fragment of a β -chain cDNA clone isolated from the BDFL cDNA library. The $V_{BDFL\beta II}$ probe is a 1.2-kb *SalI*-*BamHI* fragment from cosmid clone BDFL3.2 (compare with Fig. 2).

Methods. RNA was isolated by guanidinium isothiocyanate and CsCl gradient centrifugation^{39,40}. Blots were hybridized with oligo-labelled probes as described previously^{29,41}. The cDNA library was constructed in the λ gt11 vector as described elsewhere⁴². RNA relative molecular masses (M_r s) were determined using DNA fragments of known lengths run on the same gel. The blot obtained with the $V_{BDFL\alpha II}$ probe is overexposed to show the 1.2-kb α gene transcript.

at the end of the J gene segment. The V-region sequence is identical to the BDFL α -chain cDNA clone (Fig. 3). This identifies the αI gene as the functional allele. It is interesting to note that the V gene has an unusually long intron of 430 base pairs (bp) between the leader and the V exon. The BDFL αII allele is nonfunctional. The frame in which the V and J gene segments have been joined results in a premature termination codon at the end of the V region. The αII allele is also transcribed, although at a lower level than the αI allele, giving rise to the 1.2-kb RNA molecule (Fig. 1). We do not know why this unusually short transcript is produced.

The BDFL βI allele shows a V-D- J_β 2.5 rearrangement which deleted the $C_\beta 1$ constant-region gene (Figs 2c, 3). BDFL βI is the functional β allele because it has an open reading frame and is identical to the four β -chain cDNA clones isolated. Interestingly, this β gene contains the same V gene segment (E1) previously identified¹⁵ in a trinitrophenol (TNP)-specific helper T-cell clone restricted to I-A^d. Thus, as found previously^{18,31,32}, the same V gene segment can be used by MHC class I and class II-restricted T cells. The BDFL βII allele is characterized by a rearrangement of a V gene segment to $D_\beta 1$ and $J_\beta 1.2$ (Figs 2d, 3); the rearrangement has fused the gene segments such that a premature termination codon is encountered in the C region. On the basis of these criteria, the BDFL βII gene is nonfunctional. Also, it does not produce a β -chain transcript detectable by Northern blot analysis (Fig. 1). The BDFL βII V gene segment has not been described previously^{15,18,19}. Thus, at present, 17 V_β gene segments have been identified in the mouse genome.

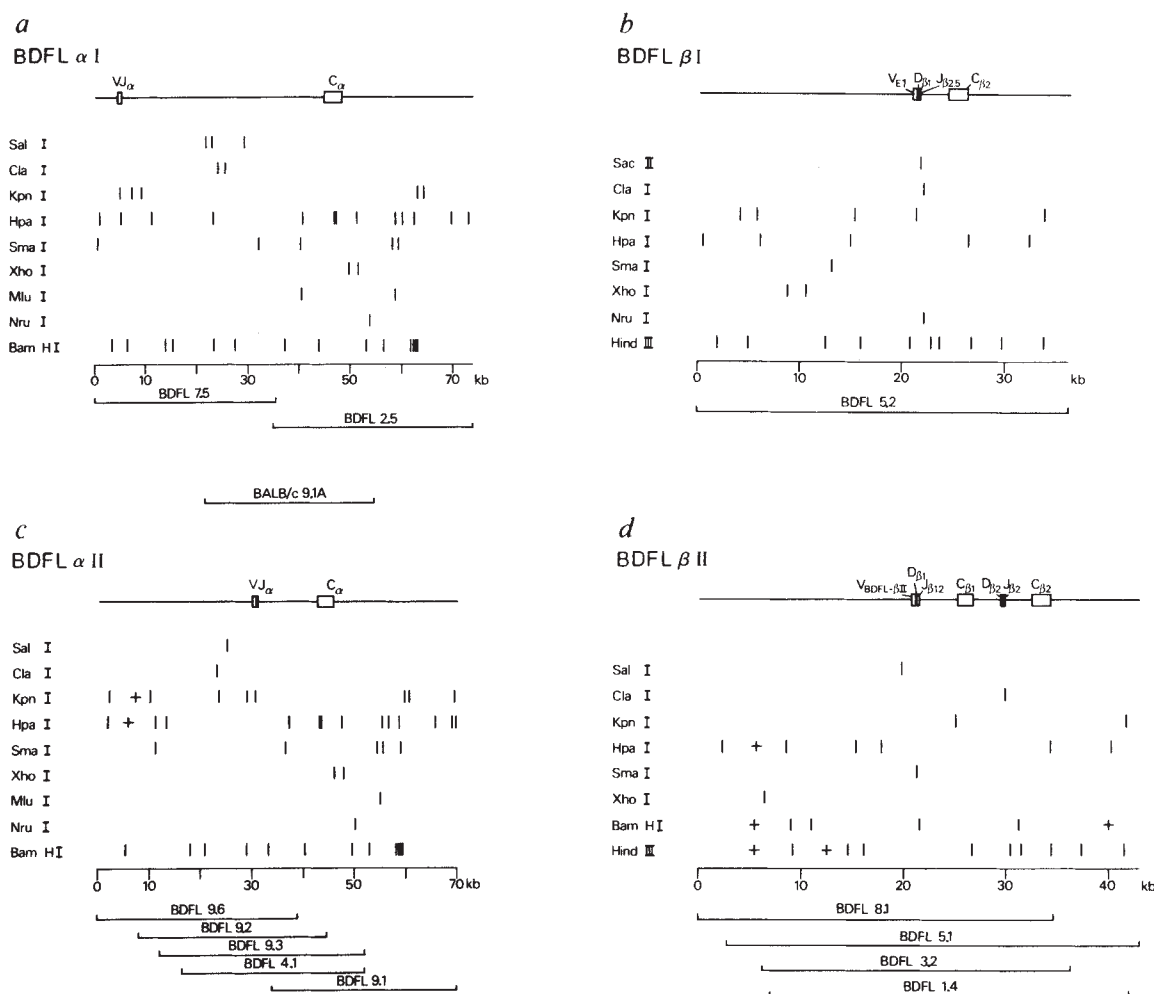


Fig. 2 Molecular maps of rearranged T-cell receptor α - and β -chain alleles from BDFL. Boxes indicate the location and approximate size of the V and C gene segments; their positions were deduced from sequence determinations (compare with Fig. 3) and by Southern blot hybridization using the V- and C-region probes described in Fig. 1 legend. The $D_{\beta}2$ - $J_{\beta}2$ rearrangement in the BDFL β I gene was deduced from smaller restriction fragments in this region, compared with germline restriction maps¹⁴, but has not been sequenced. Restriction enzyme sites are indicated by vertical bars. + Indicates the presence of unassigned sites for the same enzyme in the segment marked. *a*, The BDFL α I allele is defined by two overlapping cosmid clones derived from the DBA/2 chromosome (compare with ref. 29); as these overlap by less than 0.5 kb of DNA, a germline cosmid clone, BALB/c9.1A, was isolated from a BALB/c cosmid library⁴³ to confirm the overlap. *b*, The BDFL α II allele is defined by five overlapping cosmid clones derived from the C57BL/6J chromosome (compare with ref. 29). *c*, The BDFL β I allele is defined by one cosmid clone. No site for *Sal*I was found. *d*, The BDFL β II allele is defined by four overlapping cosmid clones.

Methods. The BDFL cosmid library was constructed and screened with oligo-labelled C_{α} , C_{β} and $V_{BDFL\alpha I}$ probes (see Fig. 1 legend) as described previously²⁹. The probe used to isolate the cosmid clone BALB/c9.1A was a 2-kb fragment extending from the left-most *Bam* HI site to the left end of cosmid clone BDFL2.5. All cosmid were analysed by restriction enzyme digestion and hybridization as described previously^{44,45}.

Transfection of functional alleles

To transfer the BDFL α I and β I alleles into another T cell, we constructed the cosmid molecule BD7 containing both genes in the same transcriptional orientation together with the neomycin-resistance gene as a positive selection marker (Fig. 4). Briefly, 30 kb of the large intervening sequence between the exons encoding the V and C regions of the α -chain were excised so that

the α gene together with the β gene would fit into the cosmid vector. The deletion in the intron ends 10 kb upstream of the C_{α} gene and therefore leaves at least one functional J_{α} gene segment²² behind. We reasoned that if the T-cell receptor α gene contains a tissue-specific enhancer element in a similar position to that in immunoglobulin heavy and light-chain genes³³⁻³⁵, it should be located downstream of the most 3' functional J_{α} gene segment and would therefore be retained in our construct. The

Fig. 3 (Opposite) Nucleotide and predicted amino-acid sequences of the T-cell receptor α - and β -chain V genes and cDNAs from BDFL. *a*, α I; *b*, α II; *c*, β I; *d*, β II. The leader (L), V, D and J regions are indicated. In BDFL α I and BDFL α II, broken arrows indicate unknown locations of boundaries and possible extra nucleotides between V and J. Dashes indicate where cDNA sequences are identical to genomic sequences. gJ α II in *b* is the sequence of the germline $J_{BDFL\alpha II}$ gene segment on cosmid clone BALB/c9.1A (compare with Fig. 2). Proposed nonamer and heptamer recognition sequences for DNA rearrangement are indicated by the respective numbers. Conserved splice donor sites at the 3' end of the J-gene segments are underlined.

Methods. The BDFL α I and BDFL β I cDNAs were excised from λ gt11 and subcloned into the *Eco*RI site of pUC9 or M13mp18. Relevant restriction enzyme fragments were isolated from cosmid clones (see below) and subcloned into pUC9 or M13mp18/19. DNA sequences were obtained using deletion subcloning⁴⁶, chemical degradation⁴⁷ and dideoxynucleotide chain termination⁴⁸ techniques. Primers used for dideoxynucleotide sequencing were either purchased (universal M13 primer) or synthesized⁴⁹. The following fragments were isolated and subcloned for sequencing (compare with Fig. 2): a 3-kb *Bam*HI from cosmid clone BDFL7.5 containing the $V_{BDFL\alpha I}$ gene (this clone was designated pV α -1); a 4-kb *Bam*HI fragment from cosmid clone BDFL9.3 containing the $V_{BDFL\alpha II}$ gene; a 1.2-kb *Hind*III-*Cla*I fragment from cosmid clone BDFL5.2 containing $V_{BDFL\beta I}$; a 1.2-kb *Sal*I-*Bam*HI fragment from cosmid clone BDFL3.2 containing $V_{BDFL\beta II}$; and a 9-kb *Bam*HI fragment from cosmid clone BALB/c9.1A containing the unrearranged $J_{BDFL\alpha II}$ gene segment.



shortened α I gene on an 18-kb fragment was then inserted, together with a 24-kb fragment containing the β I gene, into the cosmid vector pTCF containing the G418-resistance gene with simian virus 40 (SV40) promoter and enhancer sequences. In this construct the α I and β I genes retained 1 and 8 kb of 5'-flanking sequence, respectively.

The BD7 construct was then transfected by protoplast fusion into the cytolytic T-cell hybridoma SPH (ref. 36), which recognizes the hapten 3-(*p*-sulphophenyldiazo)-4-hydroxyphenylacetic acid (SP) and the K^k MHC class I molecule. The T-cell hybridoma SPH was obtained by fusion of a CBA-derived cytotoxic T cell with the AKR thymoma BW5147.

From four independent protoplast fusion experiments with BD7, we isolated 41 G418-resistant clones. Of these clones, 22 had retained the antigen and MHC-specific cytolytic activity of the recipient T-cell hybridoma (data not shown); 12 of these were analysed further.

Clone BD7-S17 transcribes transfected genes

We analysed transcription of the transfected BDFL α and β genes, since no anti-idiotypic antibody directed against the T-cell receptor of the BDFL killer cell was available. Initially, RNA dot-blot experiments were carried out on all 12 transfectants. Using the $V_{BDFL\alpha I}$ and $V_{BDFL\beta I}$ -specific probes only one transfectant (BD7-S17) showed high levels of BDFL α I gene transcription, while two transfectants (BD7-S17 and BD7-S19) showed high levels of BDFL β I gene transcription (not shown). Northern blot analysis confirmed that the BD7-S17 transfectant contained high levels of α and β transcripts hybridizing to the BDFL V-region probes (Fig. 5a). The BD7-J9 transfectant showed intermediate levels of β -gene transcripts but no α transcripts (Fig. 5a).

In contrast to the $V_{BDFL\alpha I}$ probe, the $V_{BDFL\beta I}$ probe hybridized weakly with RNA from the SPH recipient T cell (Fig. 5a), indicating that the same or a closely related V_{β} gene segment was transcribed in SPH. We therefore performed RNase protection experiments to show unequivocally that proper α - and β -gene transcripts were derived from the introduced BDFL α I and β I genes, respectively, in the transfectants.

As probes for the RNase protection experiments, we used labelled 'anti-sense' RNA transcripts derived *in vitro* from the coding strands of the $V_{\alpha I}$ and $V_{\beta I}$ genes (Fig. 5b). These probes were annealed with RNA from BDFL, SPH, BD7-S17 and BD7-J9 cells, digested with RNase and analysed on a 6% sequencing gel. As shown in Fig. 5b, BDFL and BD7-S17 RNA protected 352-nucleotide (nt) $V_{\alpha I}$ and 351-nt- $V_{\beta I}$ RNA fragments. BD7-J9 RNA protected the 351-nt $V_{\beta I}$ RNA fragment but showed no protection of the $V_{\alpha I}$ probe. SPH RNA did not protect the $V_{\alpha I}$ RNA but it (as well as RNA from the two transfectants) did protect a 291-nt fragment of the $V_{\beta I}$ RNA probe, again indicating that the same or a very similar V_{β} gene segment is transcribed in the SPH and BDFL cells. Because of the difference in size between the V_{β} fragments protected by SPH and BDFL RNA, at least the sequences around the D regions must be different in the β -chain genes of BDFL and SPH.

The results of the RNase protection experiments confirm that the introduced BDFL α I and β I genes are transcribed and spliced in clone BD7-S17 while only the BDFL β I gene is transcribed and spliced in BD7-J9. We have not found a major protected band for the 5'-untranslated regions and the leader sequences of the α and β genes, possibly because of premature termination of most of the *in vitro*-synthesized RNA molecules.

Figure 5c shows an analysis of the RNAs of the two transfectants, the donor and the recipient T cells, using an oligonucleotide probe specific for the $V_{BDFL\beta I}$ gene. With this probe transcripts of the same size were found in the transfectants and the BDFL donor cell, while no hybridization was observed with RNA from SPH recipient cells. We conclude, therefore, that the BDFL α and β gene transcripts were correctly initiated, spliced and terminated in the transfectants.

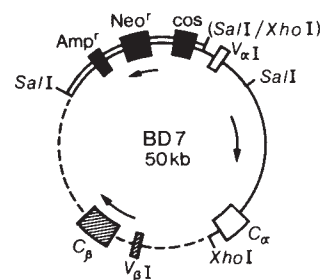


Fig. 4 Structure of the BDFL T-cell receptor gene construct. The cosmid BD7 was derived by subcloning the BDFL α I and BDFL β I genes into the cosmid vector pTCF, described in ref. 50. □, ▨: V and C genes of BDFL α I and BDFL β I, respectively. Amp^r, the β -lactamase gene of pBR322; Neo^r, the aminoglycosyl 3'-phosphotransferase gene from Tn5; cos, cos site of phage λ . Arrows indicate the orientation of transcription. The double-lined segment represents the vector; the segment indicated by a single line was derived from parts of cosmids BDFL7.5 and BDFL2.5; the segment represented by a broken line was derived from cosmid BDFL5.2 (see below). Restriction enzyme sites used in the construction are shown. Parentheses indicate that the respective sites were inactivated during the cloning.

Methods. The construction involved several subcloning steps: (1) in pV α -1 (see Fig. 3 legend) the unique *Sma*I site of the pUC9 vector was converted into a *Xho*I site by linker insertion. (2) The insert of pV α -1, excised with *Sal*I and *Xho*I, was subcloned into the *Sal*I site of the cosmid vector pTCF. One of the resulting clones, pTCFV α -C, contained the desired unique *Sal*I site 3' of the $V_{BDFL\alpha I}$ gene (the other *Sal*I site was not regenerated because of ligation of *Sal*I and *Xho*I sticky ends). (3) A 15-kb *Sal*I-*Xho*I fragment from cosmid BDFL2.5, together with a 24-kb *Sal*I-*Xho*I fragment from cosmid BDFL5.2D (derived from cosmid clone BDFL5.2 after conversion of the unique *Sma*I site into a *Xho*I site; compare with Fig. 2), was inserted into the *Sal*I site of pTCFV α -C. From the resulting clones, cosmid BD7, with one *Xho*I and two *Sal*I sites, was selected for transfection experiments. BD7 was transferred to SPH hybridoma cells by protoplast fusion as described elsewhere^{51,52}.

Specificity of BD7-S17 transfectant

To determine whether the introduced and transcribed BDFL α I and β I genes enabled the BD7-S17 transfectant to recognize the same target cells as BDFL, we tested its effect on H-2^d- and H-2^k-positive cells coupled with either fluorescein or SP. We used two different types of target cells: B-cell blasts and L-cell fibroblasts. We shall first describe the results obtained with B-cell blasts. Figure 6a shows that the BD7-S17 transfectant was able to lyse H-2^k-positive B-cell blasts coupled with SP, but could not lyse H-2^d-positive B-cell blasts coupled with FL. Thus, the transfectant had retained the cytolytic specificity of the SPH recipient cell but had not gained the specificity of the BDFL donor cell when tested on B lymphocytes.

It has been argued that T cells which display a relatively low avidity for MHC and antigen require accessory molecules (such as Lyt 2 and L3T4) to strengthen the interaction with target cells^{6,8}. The need for L3T4 to stabilize the T-cell/target cell interaction can be overcome by increasing the concentration of antigen on the surface of the target cell^{7,8}. For unknown reasons, T-cell hybridomas derived from fusion experiments using BW5147 as the thymoma parent, do not express Lyt 2 molecules³⁷. Thus, a possible explanation for the failure of the BD7-S17 transfectant to lyse B-cell blasts is that it requires Lyt 2, which was not expressed on the recipient SPH hybridoma (not shown). Indeed we found that the cytotoxic activity of BDFL cells can be blocked with anti-Lyt 2 antibodies (Fig. 6a). Thus, we hoped to overcome the requirement for Lyt 2 by using target cells expressing larger amounts of the D^d antigen.

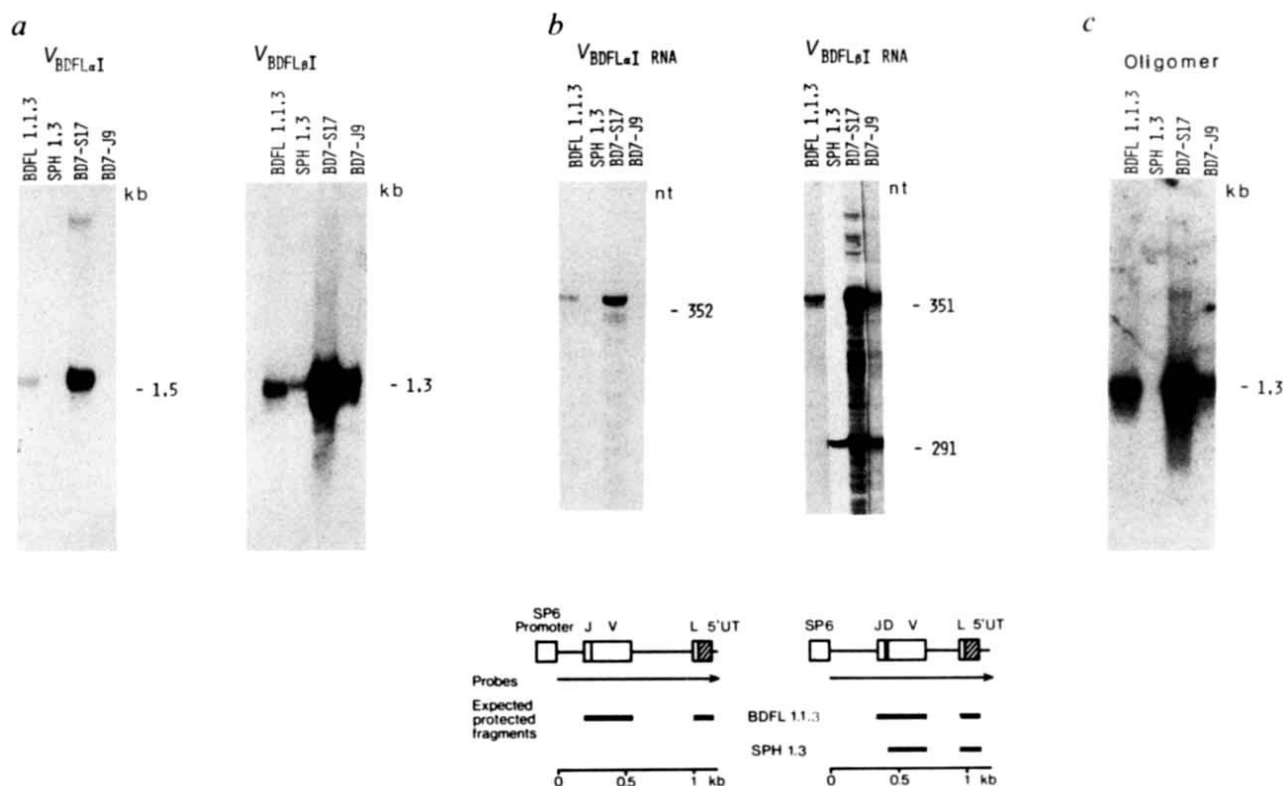


Fig. 5 Analysis of transfectants by Northern blotting and RNase protection. **a**, Northern blot analysis. Blots were obtained and hybridized with the $V_{BDFL\alpha I}$ and $V_{BDFL\beta I}$ probes as described in Fig. 1 legend. **b**, RNase protection experiment. DNA fragments containing the αI and βI V-region genes were subcloned into the pSP6 vector, labelled and hybridized with poly(A)⁺ or total RNA isolated from the indicated T cell as described elsewhere⁴³, using a commercially available RNA synthesis kit (Amersham). The $V_{\alpha I}$ RNA probe was derived from the 1.2-kb insert of a deletion subclone of pV α -1 (see Fig. 3 legend); the $V_{\beta I}$ RNA probe was derived from the 1.2-kb *Hind*III-*Cla*I fragment of cosmid clone BDFL5.2 (see Fig. 3 legend). The sizes of the protected RNA fragments were determined on 6% polyacrylamide/urea sequencing gels by co-migration with DNA sequence ladders. The sizes of the expected protected RNA fragments are shown in the diagram at the bottom. Open boxes indicate the SP6 promoter region as well as the exons of the α and β genes. **c**, Northern blot analysis. Northern blots as in **a** were hybridized with a 33-nt oligonucleotide probe specific for the 3' end of the BDFL βI V-region gene (positions 729-761 in Fig. 3). The oligomer was synthesized⁴⁹, labelled with T4 polynucleotide kinase and hybridized as described elsewhere²⁹.

Next, as target cells we used two different L-cell fibroblasts expressing high levels of either D^d or K^k MHC molecules (Fig. 6b). L cells expressing high levels of D^d antigen (L-D^d ↑; 5-7-fold more than the B-cell lymphoma A20) have been obtained by gene transfection⁸. As L cells are derived from C3H mice, the transfected L cells also express K^k antigen. L cells expressing large amounts of K^k antigen (L-K^k ↑; about the same amount as D^d and about 10-fold more K^k antigen than on L-D^d ↑) were obtained by selection (see Fig. 6 legend). Both L-cell lines were coupled with either FL or SP and tested with the donor, recipient and transfected cytotoxic T cells.

As shown in Fig. 6b, the BDFL donor T cells lysed only FL-coupled L-D^d ↑ targets well and produced some background lysis on the other targets. The SPH recipient T cells lysed the SP-coupled L-K^k ↑ targets well and showed a low level of lysis above background on SP-coupled L-D^d ↑ targets. The BD7-S17 transfectants lyse both FL-coupled L-D^d ↑ and SP-coupled L-K^k ↑ targets well; they also showed a low level of cytotoxic activity on SP-coupled L-D^d ↑ targets, as did the recipient cells.

We believe that the lysis of SP-coupled L-D^d ↑ targets by the recipient and the transfected T cells results from recognition of the endogeneously expressed K^k antigens and SP on the target cells (the specificity of the recipient). If one subtracts the background lysis of uncoupled L-D^d ↑ target cells, there is about 10% specific lysis by the transfectant and 6% specific lysis by the recipient of the SP-coupled L-D^d ↑ target cells. As these two values are not significantly different there is no evidence for the generation of receptors with mixed specificities on the surface of the transfectant. Thus, the lysis of FL-coupled L-D^d ↑

targets is the only novel specificity detected for the transfectants, which is the result expected if the MHC-restricted antigen specificity of the donor cell is expressed on the transfectants. In agreement with this result, lysis of FL-coupled L-D^d ↑ cells by the transfectant was significantly inhibited by anti-D^d antibodies (Fig. 6c).

The inability of the BD7-S17 transfectant to lyse FL-coupled B-cell blasts expressing 'normal' levels of D^d can be explained by the absence of Lyt2 molecules on the transfectant. Apparently, and in agreement with previous findings^{7,8}, this deficiency can be compensated for by over-expression of target antigens. Transfer of the recently isolated Lyt2 gene³⁸ into BD7-S17 should allow us to test this explanation.

The low killing efficiency of the BD7-S17 transfectant might also be explained in other ways: for example, other cell surface molecules (possibly the T-cell receptor-related γ -chain of the donor cell) might be important in stabilizing the interaction with target cells. Also, a low concentration of the donor-derived α/β heterodimer on the transfectant could explain the killing inefficiency. The latter possibility can be tested by immunoprecipitation and two-dimensional gel electrophoresis to distinguish between the various α - and β -chains.

In contrast to BD7-S17, the BD7-J9 transfectant, transcribing the BDFL βI but not the αI gene, does not lyse FL-coupled L-cell targets expressing high levels of the D^d class I molecule. Thus, it is unlikely that the β -chain alone can transmit specificity as tested in our system. We have not tested any transfectants expressing the BDFL αI chain alone. Therefore, we conclude that in our system the T-cell receptor α - and β -chains are

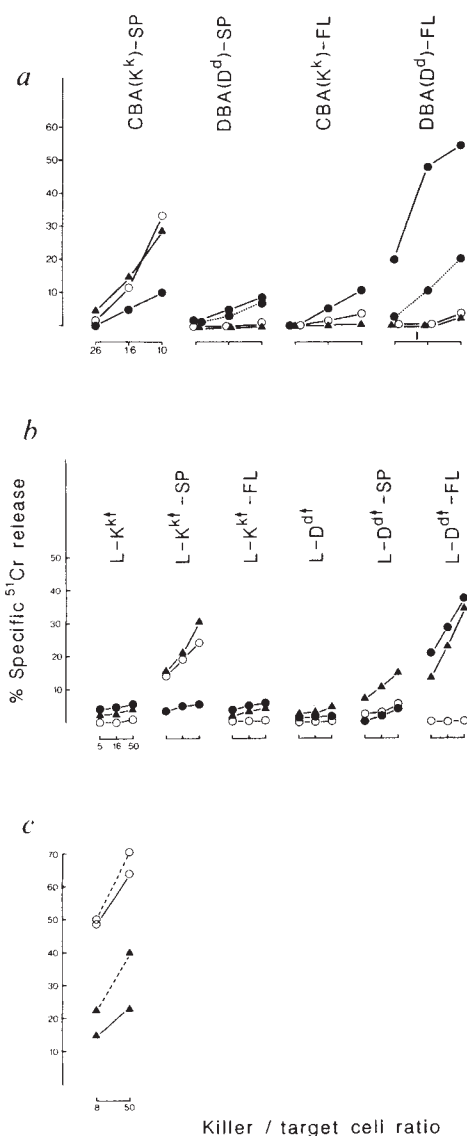


Fig. 6 *a*, Cytolytic activity of SPH (recipient), BDFL (donor) and BD7-S17 (transfectant) T cells on lipopolysaccharide (LPS)-stimulated B-cell blasts coupled with either the hapten 3-(p-sulphophenyldiazo)-4-hydroxy-phenylacetic acid (SP) or fluorescein (FL). ●—●, BDFL 1.1.3 (FL+D^d); ●—●, BDFL 1.1.3 plus anti-Lyt 2 antibodies; ○, SPH 1.3 (SP+K^k); ▲, BD7-S17. The target LPS blasts were labelled with ⁵¹Cr and specific ⁵¹Cr release was determined in a ⁵¹Cr-release assay⁵⁴. For inhibition with anti-Lyt 2 antibodies (53-6.72; 100 μg ml⁻¹), cytolytic T cells were incubated with purified antibodies for 15 min before addition of target cells. *b*, Cytolytic activity of SPH (recipient, ○), BDFL (donor, ●) and BD7-S17 (transfectant, ▲) cells on L-cell fibroblasts coupled with either SP or FL. L-K^k cells are LS5 cells (Flow Laboratories) derived from the L929 cell line; they were selected for their high expression of K^k antigens. L-D^d cells are Ca 25.8.1 cells transfected with a D^d class I MHC gene (provided by B. Malissen); this cell line expresses about one-tenth of the level of K^k antigens expressed by the LS5 cells. As assessed by fluorescence-activated cell sorter analysis, the amount of K^k antigens expressed by the LS5 cells is slightly higher than the amount of D^d antigens expressed by the Ca 25.8.1 cells (as tested using monoclonal antibodies H0-100-5/28 (anti-K^k) and 34-7-23 (anti-D^d) at a concentration of 100 μg ml⁻¹). *c*, Inhibition of cytolytic activity of BD7-S17 (transfectant) T cells by preincubation of L-D^d cells with anti-D^d (34-7-23) antibodies (100 μg ml⁻¹) for 15 min before addition of the cytolytic T cells. As a control we used FLH cells whose specificity is not restricted by polymorphic MHC determinants⁵⁵. ○—○, FLH; ○—○, FLH plus anti-D^d; ▲—▲, BD7-S17; ▲—▲, BD7-S17 plus anti-D^d.

sufficient to transfer the MHC-restricted antigen specificity to recipient cells.

Conclusion

These results provide the first definite proof that the T-cell receptor α - and β -chain genes can transmit a functional specificity from one cytotoxic T cell to another. The ability to transfer T-cell receptor specificities by gene transfection opens up new ways of studying, by *in vitro* manipulation, the molecular interactions that enable T cells to recognize both MHC and foreign antigen, genetic elements important for T-cell receptor gene regulation and the development of the T-cell receptor repertoire during ontogeny.

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Very Large Array observations of rapid non-periodic variations in OJ 287

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Time variations in compact extragalactic objects can be used to constrain both their emission mechanisms and models for their geometry. In particular, periodic variations constrain the popular black hole model for these sources^{1,2}. There have been many reports of the presence (and absence) of both periodic³⁻⁸ and non-periodic⁹ variability in the BL Lacertae object OJ 287 = 0851 + 202, from the optical and infrared through centimetre radio wavelengths (Table 1). The apparent consistency of the reported periodicity near $P = 15.7$ min and the absence of variations in other sources observed in the same way lends some credence to these variations, even though they are only a few per cent of the mean intensity. We observed OJ 287 with the Very Large Array (VLA) at 5, 15 and 22 GHz several times in early 1983. The 5-GHz data, taken coincident with the 22-GHz observations of Valtaoja *et al.*⁸, show OJ 287 to have decreased in flux by about 1.8% in an irregular way over a span of about 7 h, and to have fluctuated by about 0.5% on a timescale of about 15 min. Brightness temperatures derived from causality constraints range between 10^{16} and 10^{20} K. Fourier spectra of the time series were analysed using a novel one-dimensional complex CLEAN algorithm to remove the effects of the data sampling. At 5 GHz there was no harmonic component to the intensity of OJ 287 which exceeded 0.1% of the mean for any period between 80 and 5,000 s. The limits at 15 and 22 GHz were 2% and 0.8%, respectively.

The 5-GHz observations were made in the standard hybrid C-D configuration of the VLA over the UT interval 1983 May 23.859 to 1983 May 24.171, using both right and left circular polarizations in a 50-MHz bandwidth centred at 4.8851 GHz. To provide an internal check on the observations and to give continuous time coverage on each object, the VLA was divided into two independent subarrays which observed OJ 287 and the calibrator 0839 + 187 for 6 min 20 s each in two alternating sequences 180° out of step (except for a short period during which the subarrays inadvertently became synchronized). This calibrator is a steep-spectrum, unresolved source (class P in the VLA nomenclature) which lies 3.2° away from OJ 287. The subarrays had 13 and 14 antennas, selected to occupy alternate stations of the array so that the two subarrays had nearly-identical, interlocking spatial configurations. Each subarray also

Table 1 Suggested periodicities in OJ 287

Previously-reported harmonic amplitude (%)						
Period (min)	5 GHz	15 GHz	22 GHz	37 GHz	Optical	Ref.
42.2	—	—	—	—	2.2	6
40.5	—	—	—	—	4.0	4
39.2	—	—	—	—	0.6	3
22.8	—	—	—	—	2.8	6
15.7	—	—	0.5	0.5-5.0	D	7, 8
13.0	—	—	0.4	D	—	7, 8
12.0	—	—	—	—	0.6	6
19.0-148.0	—	—	—	—	<0.15	5
Upper limits from this work (%)						
Period (min)	5 GHz	15 GHz	22 GHz	37 GHz	Optical	Ref.
1.3-83.3	<0.1	<2.0	<0.8	—	—	—

The symbol D signifies a reported detection at an unspecified level.

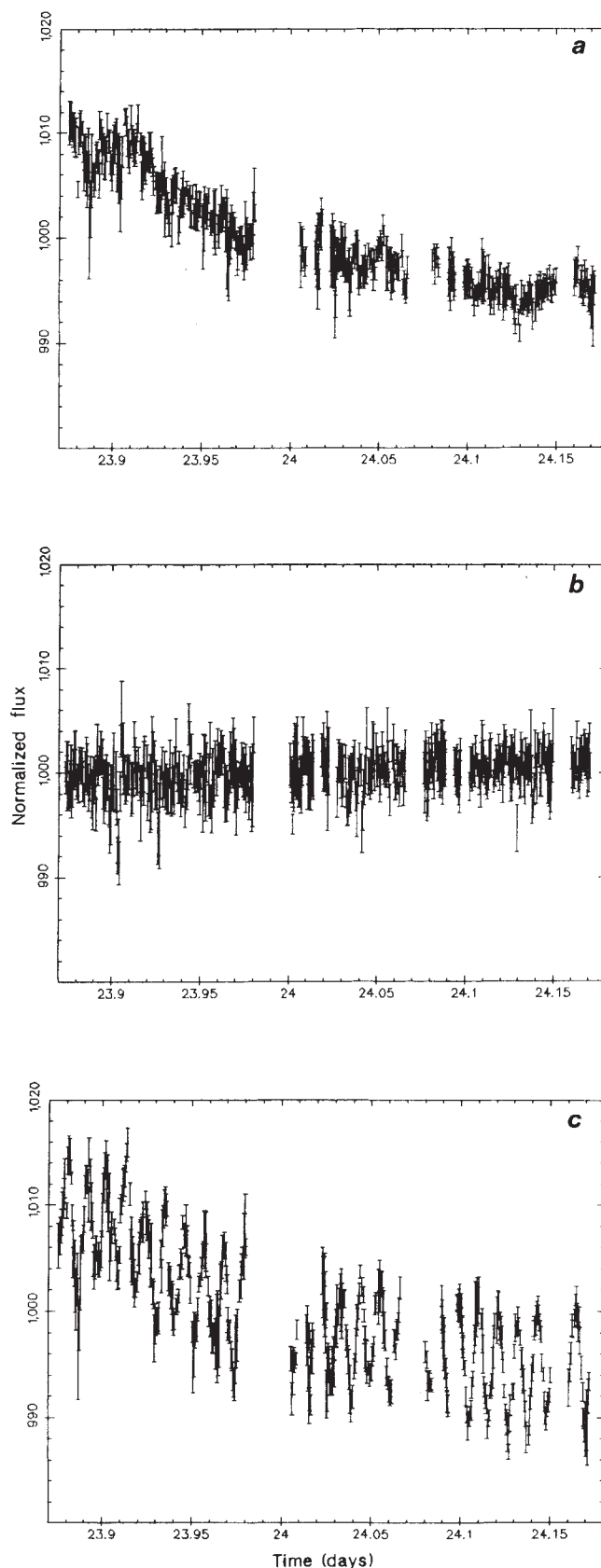


Fig. 1 Normalized flux densities of OJ 287 (a) and 0839 + 187 (b) obtained at 5 GHz by the VLA on 23-24 May 1983, combining the two subarrays and averaging the two polarizations. Artificial data consisting of an amplitude $p = 0.5\%$ harmonic component with period 15.7 min superimposed on our actual data for OJ 287 are shown in c.

made two short observations of 3C286 (assumed flux 7.41 Jy) to determine the flux scale, and two of OJ 287 in pointing mode to check the pointing of all the antennas. The observations took place in the afternoon, with scattered clouds and winds of $<6 \text{ m s}^{-1}$. The phase stability on the longest spacings ($\sim 2 \text{ km}$) was $\sim 20^\circ$ r.m.s. over 6 min; shorter baselines had significantly better phases.

The visibility data for each subarray and polarization were corrected for the amplitude and phase gains of each antenna using the standard methods, as detailed below. Despite stringent standards, only a few data were edited out. By comparison with 3C286 the flux of 0839+187 was found to be 0.943 Jy, with an internal agreement among antennas of better than 1%. The average flux of OJ 287 was 4.47 Jy. The amplitude gain of each antenna as a function of time was derived on the assumption that 0839+187 had a constant flux; these scan-averaged gains were applied to observations of both 0839+187 and OJ 287 by linear extrapolation over approximately 13 min. The phase gain of each antenna as a function of time was determined by assuming that each source was unresolved and calibrating each source on itself using visibility data vector-averaged over 20 s. A gain table with 1-min intervals was used in all cases. These procedures effectively eliminate systematic pointing errors, atmospheric attenuation, instrumental gain drifts and baseline errors as possible causes of any apparent flux variations in OJ 287.

The same program that solves for the gains (ANTSOL) was then used to extract the time behaviour of OJ 287. The code assumes that the flux S of OJ 287 is a constant, and solves for the amplitude gain of each antenna $g_i(t)$ required to adjust the visibility data to make this so. Since the visibilities were amplitude-calibrated for a nominal flux of 4.47 Jy, the resulting power gain $g_i^2(t)$ of each antenna measures the ratio $[S(t)/4.47 \text{ Jy}]$; the normalization is such that a flux of 4.47 Jy yields a power gain of 1,000. This procedure was applied to both OJ 287 and the calibrator, using 40-s vector averages of the data. Errors in the gain calibration are expected to be associated almost exclusively with the individual antennas, so the degree of internal agreement among the fluxes determined from the different antennas provides valuable information on the accuracy of the measurements.

Bad data points were removed from the time series of each source by excluding those points which deviated from 'provisional means' by more than three 'provisional standard deviations'. A provisional mean for each 40-s segment was found by averaging the 2/3 of the individual antenna data points nearest to their median, and the provisional standard deviation was computed from the 2/3 of the points nearest to it. Only 1,809 of 12,167 data points were removed for OJ 287, and 1,721 of 12,509 for 0839+187. The filtered data were re-scaled so that the overall average for each antenna was 1,000, and from these a mean flux and standard deviation of the mean were produced every 40 s for each subarray and polarization separately. Figure 1a and b shows the resulting flux histories of OJ 287 and 0839+187 obtained by combining the two subarrays and averaging the two polarizations. The breaks occur where there were interruptions in the data-taking. For comparison, artificial data consisting of a 0.5%-amplitude sinusoidal component with period 15.7 min = 943 s (similar to the variation reported at 22 GHz by Valtaoja *et al.*⁷ for the same day) added to our actual data for OJ 287 are shown in Fig. 1c.

Errors in these fluxes can arise from numerous sources. The 40-s polarization-averaged mean fluxes for the calibrator showed r.m.s. fluctuations of 0.27% about the long-term average flux; as the thermal noise for each subarray for each 40-s mean is expected¹⁰ to be $\sim 1.1 \text{ mJy}$, corresponding to 0.11% r.m.s. for 0839+187, there were other sources of amplitude noise. This conclusion is supported by the fact that the fractional 'noise' for OJ 287 was nearly as great as for 0839+187 despite the fact that OJ 287 is a factor of five stronger. Our estimates of various sources of error in flux determination are summarized in Table

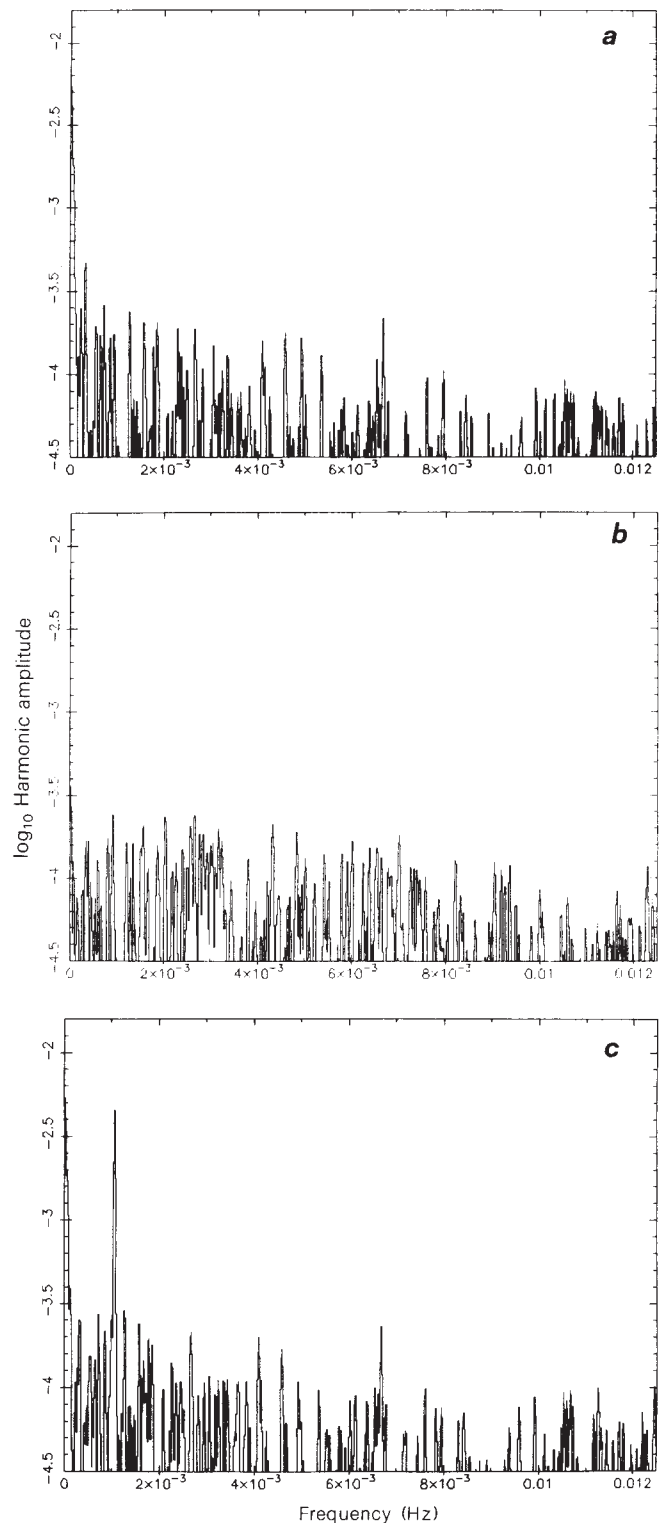


Fig. 2 Harmonic amplitudes of OJ 287 (a), 0839+187 (b), and the artificial data for OJ 287 (c), derived from the time series of Fig. 1, on a logarithmic scale. A conservative upper limit on harmonic variability of OJ 287 with periods between 80 s and 83 min is an amplitude of 0.1% of the mean.

2. The pointing observations showed an array-averaged systematic pointing error of roughly 18 arc s (root sum square of both axes), probably reflecting the well-known effect of sunlight on the antenna mounts. The pointing errors of the individual antennas varied about this mean with an r.m.s. of 23 arc s. While these

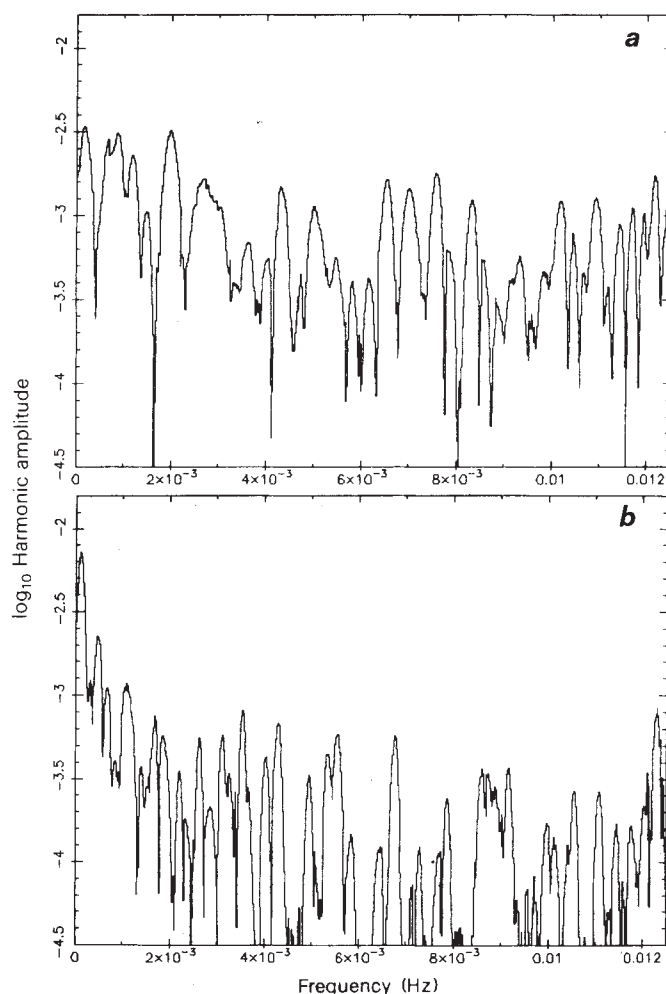


Fig. 3 Harmonic amplitudes of OJ 287 at 15 GHz on 21 May 1983 (a) and at 22 GHz on 17 March 1983 (b) on a logarithmic scale. Conservative upper limits to harmonic variability with periods between 80 s and 83 min are amplitudes of 2% and 0.8% of the mean, respectively.

errors slightly reduce the antenna gains (typically by 0.3%), they are dominated by effects that vary over hours in time and radians on the sky; thus the resulting gain errors are almost totally removed by the calibration. We estimate that short time-scale (minutes) pointing errors, which cannot be corrected, were less than 15 arc s; numerical simulations show that at a mean offset of 18 arc s from the beam axis these contribute r.m.s. amplitude fluctuations $<0.18\%$ per antenna, or 0.05% per subarray. Assuming a 1-km pathlength through a fair-weather cumulus cloud which covers an entire subarray for minutes, the resulting variations in opacity could give amplitude fluctuations as large as 0.2% per subarray¹¹. Rapid variations of atmospheric phase can erratically reduce apparent fluxes by decorrelation of the fringes; to reduce the flux measured by one antenna by 0.1% requires a phase change of 9° over 20 s, which seems implausible for our observing conditions. 'Closure' errors arise from limitations in the assumption that the gains are strictly antenna-based; typical values are 1% in amplitude for each correlator. For the mean fluxes these errors should be reduced (by roughly the square root of the total number of correlators) to $<0.1\%$. Rapidly varying errors in the antenna delays can also produce apparent amplitude fluctuations. Although not yet well understood, these variable delay errors are believed to be <0.3 ns, which, combined with constant delay offset errors of ~ 1 ns, could result in a reduction of $<0.3\%$ in apparent flux

Table 2 Sources of error in VLA 5-GHz flux measurements

Source of error	Magnitude of error	
	One antenna	Subarray of 12
Thermal noise of system (2 pols, 40 s)	2.5 mJy	0.8 mJy
As fraction of 0839 + 187	0.27%	0.08%
As fraction of OJ 287	0.06%	0.02%
Rapidly varying pointing errors ($\sim 15''$)	0.2%	0.05%
Opacity of a 1-km fair-weather cumulus cloud drifting over the whole array	0.2%	0.2%
Phase winding within 20-s visibility averaging (at 10° per min)	0.01%	0.00%
Correlator closure errors (1% in amplitude)	0.2%	0.09%
Rapidly varying delay errors of <0.3 ns	$<0.3\%$	$<0.09\%$
Finite resolution of VLA AGC system	$<0.1\%$	$<0.03\%$
ANTSOL printout accuracy	0.05%	0.01%
Maximum root sum square error		
For 0839 + 187	0.54%	0.26%
For OJ 287	0.47%	0.25%

per antenna and $<0.09\%$ for a subarray. Finally, errors arising from the automatic gain control (AGC) system of the VLA and from certain approximations used in our reductions add a combined error that we estimate is $<0.03\%$ for each subarray flux measurement. We conclude that the precision of an individual 40-s subarray flux measurement is limited by known random and systematic errors to $\sim 0.3\%$, in good agreement with the calibrator observations.

The time series for OJ 287 showed a decline of $\sim 1.8\%$ over the ~ 7 h of data. This change corresponds to a formal timescale of $\tau = S/|dS/dt| \leq 16$ days, a short but not atypical interval for actively variable sources. The more rapid 'dip' of $\sim 0.5\%$ which occurred over ~ 15 min near $t = 23.89$ days has $\tau \leq 2$ days. These variations were seen in both circular polarizations of all antennas in both subarrays and appear highly significant in terms of the known errors. We feel, therefore, that these variations were real, but experience using the VLA to measure fluxes to an accuracy of $<1\%$ is as yet too limited to provide absolute certainty.

Simple causality arguments which limit the size R of a source to $R < c\tau/(1+z)$ may be combined with the angular size distance of OJ 287 (770 Mpc for $z = 0.306$, $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and $q_0 = 0.5$) to infer lower limits to the brightness temperature of 1×10^{16} K for a 1.8% change in 7 h and 6×10^{17} K for a 0.5% change in 15 min. If the part of the source which contributes the variable flux δS is physically separate from that which provides the steady flux S , the brightness temperature of the varying region is increased by a factor as large as $(S/\delta S)$, leading here to 6×10^{17} and 1×10^{20} K, respectively. Such extraordinary inferred brightness temperatures would seem to require either a coherent emission mechanism, unprecedented Doppler boosting, or strong centimetre-wavelength scattering.

In order to search for periodic variations in the intensity of OJ 287, separate temporal spectra for each subarray and polarization were constructed by discrete complex Fourier transform after subtraction of the means. A fast Fourier transform was not used in order that the data need not be binned. These spectra are sensitive to harmonic fluctuations with periods between 1 data span (~ 7 h, corresponding to $\sim 4.0 \times 10^{-5}$ Hz) and twice the sample period (80 s, corresponding to 1.25×10^{-2} Hz, the Nyquist frequency). The effects of the data window (artefacts and distortion due to the sampling of the data) were removed by performing a one-dimensional complex CLEAN deconvolution analogous to the (two-dimensional) technique widely used on aperture synthesis images (refs 12, 13 and D.H.R., J.L. and J.W.D., in preparation). Since half of the power in this spectrum occurs at negative frequencies, a pure harmonic signal with amplitude a fraction p of the mean appears as a spectral ampli-

tude of $p/2$ in the positive-frequency domain. However, for clarity we use the harmonic amplitude p in our discussion.

The harmonic amplitudes for OJ 287 and 0839+187 are shown in Fig. 2a, b; these are derived from the averages of the separate CLEANed spectra of the two polarization-averaged subarrays. The Fourier spectrum of the artificial data of Fig. 1c shows the anticipated amplitude of $p = 0.5\%$ at the induced period of 943 s (Fig. 2c); this shows how easily we would have detected such a signal, even in the presence of the intrinsic, low-frequency, non-periodic fluctuations and the random amplitude noise. The spectrum of the calibrator has no peak above $p = 0.039\%$. The spectrum of OJ 287 shows no peak near the previously reported period of 943 s (1.06×10^{-3} Hz). Because the noise is not gaussian, and because the detectability of a signal depends on the sampling as well as the signal-to-noise ratio, it is not possible to give a simple criterion for the smallest detectable harmonic signal. Thus we determined upper limits by processing artificial data sets with decreasing harmonic amplitudes until the signal was lost. At 5 GHz this conservative limit is 0.1% of the mean.

A significant broad component at the level $p = 0.53\%$ is seen for OJ 287 in the lowest-frequency bins (4×10^{-5} – 1.5×10^{-4} Hz), corresponding to periods between ~ 2 and ~ 7 h. This reflects the power in the nonperiodic fluctuations apparent in Fig. 1a. For 0839+187, such components (if present) should have been removed by the calibration; they appear in the spectrum (Fig. 2b) at a level of only $p = 0.04\%$.

Due to scheduling constraints, poor weather, computer failures, and severe instrumental problems only a limited number of useful data were obtained at higher frequencies. Observations at 15 GHz in the C–D array on 21 May 1983 yielded ~ 5 h of rather poor data. At 22 GHz, 2.5 h of data were obtained in the C array on 17 March 1983. The resulting power spectra are shown in Fig. 3, from which we place conservative upper limits on periodic variability of OJ 287 at 15 and 22 GHz of 2% and 0.8% respectively. With more time and better luck these figures could be substantially reduced.

Our observations lend no support to the reports of periodic variability in OJ 287. At 22 GHz our upper limit for periodicity is substantially less than the reported 'consistent' $\sim 2.5\%$ amplitude variation found at Metsahovi during 1981 to 1983, but remains (barely) compatible with the 0.5% amplitude found on a single day with the 140-foot telescope at the National Radio Astronomy Observatory^{7,8}. This latter observation occurred simultaneously with our 5-GHz observations; if the periodic variation reported at 22 GHz is correct, then our upper limit implies that the spectrum of the varying component must rise remarkably steeply ($\alpha > +1.7$ where $S_\nu \propto \nu^\alpha$) between 5 and 22 GHz. Only prolonged flux monitoring of very high sensitivity and reliability can ultimately decide whether periodic variations occur in OJ 287.

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Are cometary nuclei primordial rubble piles?

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Whipple's¹ icy conglomerate model for the cometary nucleus has had considerable success in explaining a variety of cometary phenomena such as gas production rates and nongravitational forces. However, as discussed here, both observational evidence and theoretical considerations suggest that the cometary nucleus may not be a well-consolidated single body, but may instead be a loosely bound agglomeration of smaller fragments, weakly bonded and subject to occasional or even frequent disruptive events. The proposed model is analogous to the 'rubble pile' model suggested for the larger main-belt asteroids², although the larger cometary fragments are expected to be primordial condensations rather than collisionally derived debris as in the asteroid case. The concept of cometary nuclei as primordial rubble piles is proposed as a modification of the basic Whipple model, not as a replacement for it.

The growing recognition of the importance of comets in understanding the origin of the Solar System has resulted in far more intensive study and observation of them in recent years. Observations made at greater heliocentric distances, at higher resolutions and with new techniques have occasionally produced results which cannot be easily explained with the conventional icy conglomerate or 'dirty snowball' model. Although Whipple did not specifically address the subject of the large-scale structure of the cometary nucleus, a single, well-consolidated body was clearly implied. It is worthwhile to ask how the Whipple model could be modified in order to explain these new and different phenomena.

Radar observations of comet IRAS-Araki-Alcock (IAA)³ indicated that the nucleus was "very rough on a scale larger than the radar wavelength" (3.5 and 12.9 cm), that there was a substantial departure from sphericity, and that 25% of the radar cross-section came from a slowly expanding fan of debris around the nucleus. This last observation, in particular, suggests large particles (dimensions greater than the radar wavelength) breaking free of the nucleus, despite the fact that such large particles are expected to be too heavy to be lifted off the nucleus surface by the low activity observed in IAA. The radar observations also showed evidence of 'radar-bright' features on the surface of the rotating nucleus, which might be interpreted as facets on a highly irregular surface topography.

Additional observations give evidence for substantial, slow-moving debris clouds associated with cometary nuclei. Comet Bowell at large heliocentric distance showed a nearly constant coma with expansion velocity $< 1 \text{ m s}^{-1}$, and an absence of sub-millimetre particles^{4,5}. IRAS (Infrared Astronomy Satellite) images have revealed dense meteoroid streams in the orbits of some short-period comets⁶, an unexpected result because meteoroid streams were presumed to be too dispersed to be detectable with IRAS. Observations of disturbances in the solar wind from the Pioneer Venus spacecraft have been correlated with possible outgassing from a debris stream in the orbit of asteroid 2201 Oljato⁷, an Apollo asteroid which is a likely candidate for an extinct cometary nucleus.

Whipple⁸ has suggested that a pair of outbursts in comet P/Holmes may be the result of a grazing collision followed by impact of an orbiting nucleus satellite. Doublet or multiple craters on planetary surfaces⁹ may be the result of cometary nuclei which disintegrated at the planet's Roche limit, or were already discrete 'swarms' of particles (or satellites) before they

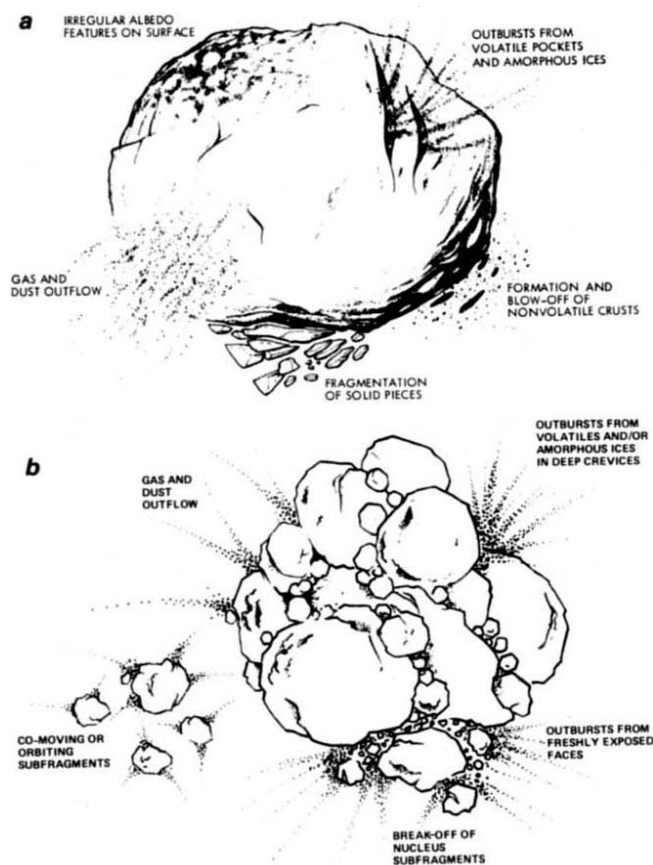


Fig. 1 *a*, Conceptual illustration of the conventional (icy-conglomerate) cometary nucleus model showing a single, well-consolidated nucleus and some sublimation-related processes which may be acting on the nucleus surface. *b*, Conceptual illustration of the rubble-pile model showing a nucleus made up of many smaller fragments, weakly bonded by local melting at contact interfaces, and perhaps surrounded by orbiting debris. A range of surface processes somewhat different from those shown in *a* nonetheless lead to similar observed phenomena from the comet.

came within the planet's sphere of influence¹⁰. de Pater *et al.*¹¹ found evidence for 'clumpy' OH emission in the coma of comet Halley, possibly indicative of multiple sublimation sources.

These observations all suggest the existence of large fragments broken off the cometary nucleus, too large to be rapidly swept into different orbits by radiation pressure or Poynting-Robertson effects. The former process affects micrometre-size dust particles, the latter centimetre-size particles. Thus, the fragments appear to have dimensions ranging from tens of centimetres to perhaps hundreds of metres, and are either orbiting or co-moving with the cometary nuclei.

The very weak gravity fields of cometary nuclei, with an escape velocity of the order of 2.2 m s^{-1} for a 3-km-radius nucleus like that of Halley, are not expected to be able to retain large fragments in near-nucleus orbits. Differential keplerian motion should slowly disperse unbound large particles into different solar orbits. On the other hand, the large particles may behave according to the 'fluidized bed' model of Brin¹², being briefly levitated off the nucleus by outbursts or spin-induced fragmentation, before settling back slowly in the very weak gravity field. The larger particles might even 'bounce' several times before coming to rest on the nucleus, perhaps mimicking the behaviour of the nucleus satellite suggested for P/Holmes.

Figure 1*a* is a conceptual illustration of an icy-conglomerate cometary nucleus¹³, showing some sublimation-related processes which may be acting on the nucleus surface. The nucleus

is envisaged as a well-consolidated, irregularly shaped, heterogeneous mixture of ice and dust, rotating about some rapidly precessing axis as it loses material through a variety of surface processes. Figure 1*b* is a similar conceptual illustration of the icy-conglomerate rubble-pile model proposed here. Although many of the proposed surface processes are the same as in Fig. 1*a*, some of the others vary somewhat in detail; nevertheless, they will produce similar observed phenomena, and will perhaps aid in explaining some of the anomalous observations described above.

One of the strongest arguments for the rubble-pile model comes from observations of cometary disruption events; that is, comet splitting. Observed statistics show that $\sim 10\%$ of dynamically new comets from the Oort cloud split on their first return, compared with $\sim 4\%$ of all long-period comets, and only about 1% of short-period comets¹⁴. The disruption events appear to occur randomly with respect to time of perihelion, heliocentric distance and distance from the ecliptic plane. It appears that disruption is associated with some intrinsic instability within the nucleus, so that comets which are likely to split do so rather rapidly, while those disinclined to split are able to survive for many returns, as is necessary if they are to evolve dynamically to short-period orbits.

Observations of splitting events shows that they typically involve small fragments (compared with the total nucleus dimensions) breaking off from the main nucleus, and that multiple fragments are occasionally produced¹⁵. Comet West in 1976 was one of the best examples of this, ejecting three subfragments near perihelion, two of which persisted for 5 months or more. The separation velocities of the fragments are typically very small, on the order of 1 m s^{-1} or less, and outbursts are frequently associated with the splitting event.

It seems logical that disruption events may result from the breaking-free of weakly bonded fragments from the nucleus as the comet approaches the Sun and the thermal wave penetrates to volatile ices at fragment interfaces. Depending on the exact nature of the nucleus, its composition and its past thermal evolution, the solar energy may penetrate either rapidly or slowly, resulting in largely random observed times for disruption events relative to perihelion.

Also, the changing moments of inertia of the nucleus as material is lost asymmetrically from its surface will result in rapid precession of the rotation axis, centrifugally stressing fragment bonds that may not have been stressed on an earlier apparition, or even earlier in the same perihelion passage.

Is the primordial rubble-pile model consistent with the generally accepted picture of cometary origin? Comets are believed to have formed as icy planetesimals in the outer planetary region^{16,17}, certainly in the Uranus-Neptune zone¹⁸, and probably also in an extended solar accretion disk extending out to hundreds of AU or more¹⁹. Greenberg *et al.* have recently reviewed the topic²⁰.

In the basic model, icy grains settle to the equatorial plane of the protosolar nebula. Larger grains settle more rapidly and grow as they settle, reaching sizes of up to tens of metres²¹ by the time they arrive at the central plane. Some additional growth may occur before the density of material in the plane becomes high enough to result in gravitational instabilities²², which force the disk to disintegrate into many bodies with typical dimensions of 1–10 km.

Some authors have suggested that comets formed at larger solar distances, in subfragments of the protosolar nebula or perhaps even pre-solar interstellar clouds^{23,24}. Although the low densities in interstellar clouds would seem to preclude this idea, what follows would be just as true for those models as for the nebula-accretion-disk hypothesis described above.

The accretion and collapse occur at low velocity, as no large bodies have yet grown in the accretion disk to stir up the velocities of the nebular condensates and/or planetesimals. The total gravitational potential energy for assembling a 3-km-radius

comet from infinity is $1.5 \times 10^4 \text{ erg g}^{-1}$, not enough to raise the mean temperature of the nucleus by 0.01 K.

Low-velocity collisions between fragments are likely to result in some local crushing and melting at the contact surfaces, helping to form a bond at these points, but are unlikely to destroy the individual structure of the fragments. The resulting nucleus would have substantial voids between fragments, and would preserve the great range of fragment sizes.

Storage at large solar distance in the Oort cloud²⁵, the lack of substantial internal heat sources²⁶, and the very weak cometary gravity field would probably result in little change in this nucleus structure over the history of the Solar System. The comet would be too cold and too small for any substantial mass migration.

In many ways the proposed model reflects on a macroscopic scale the proposed microscopic model of Daniels and Hughes²⁷, in which the nucleus is a heterogeneous aggregate of ice and dust grains with substantial voids. That model has also been scaled up²⁸ to give an aggregate model for cometary nuclei very similar to the rubble-pile model proposed here. There is also some similarity with an earlier comet model by O'Dell²⁹, although he emphasized the possibility that comets might be gravitationally bound collections of nonvolatile cores with volatile coatings acquired at large solar distance. The view expressed here is that comets are agglomerations of smaller icy-conglomerate bodies, weakly bound by local melting at contact surfaces.

The question arises as to whether the spacecraft flybys of comet Halley in March 1986 will be able to discriminate between the conventional nucleus model and the rubble-pile model proposed here. Even with high-resolution imaging, the rubble-pile nature of the nucleus may be hidden by the collection of cometary debris near contact interfaces, hiding the true nucleus structure.

The resolution of the narrow-angle cameras of the spacecraft Vega 1 and 2 at 10^4 km distance is expected to be 400 m per line pair, able to discriminate only the gross structure of the nucleus. It is likely that the Halley nucleus may still appear rough at this scale, but individual nucleus fragments with typical dimensions of tens of metres could not be resolved. (The option to send Vega 2 to a closer flyby distance may make resolution of large fragments possible.)

On the other hand, the Giotto spacecraft is targeted for a 500-km flyby with an expected camera resolution of 10 m. This certainly might be able to discriminate individual subfragments of the Halley nucleus. But if Halley is actively ejecting subfragments of metre or even centimetre size, in greater quantity than would be expected from extrapolating our knowledge of micrometre-size particles to the centimetre or greater size range, then it is unlikely that Giotto will survive to reach its minimum approach distance.

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Deuterium in the outer Solar System: evidence for two distinct reservoirs

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We have just completed a series of determinations of the $\text{CH}_3\text{D}/\text{CH}_4$ ratio in the atmospheres of Saturn^{1,2}, Titan^{3,4} and Uranus^{1,3}. These results, coupled with the work of other investigators, suggest that the Solar System contains at least two distinctly different primordial reservoirs of deuterium: that contained in gaseous hydrogen and that contained in the volatiles which have been maintained at low temperatures or isolated from hydrogen; for example, trapped in cold, solid material. Both of these reservoirs were established before the formation of the Solar System.

Figure 1 summarizes the D/H ratios observed in the methane component of the atmospheres of each of the outer planets and Titan. The values shown are derived from measurements of the $\text{CH}_3\text{D}/\text{CH}_4$ ratio according to stoichiometry: $\text{D}/\text{H} = 1/4[\text{CH}_3\text{D}/\text{CH}_4]$. All of our work used the $3\nu_2$ overtone band near $1.6 \mu\text{m}$ observed with the Fourier transform spectrometer on the 4-m telescope at Kitt Peak National Observatory¹⁻⁵. We have supplemented our results with the average equivalent value of D/H in methane for Jupiter derived from ground-based ($\text{D}/\text{H} = (3.6 \pm 0.6) \times 10^{-5}$) (refs 6, 7) and Voyager ($\text{D}/\text{H} = (3.7 \pm 1.8) \times 10^{-5}$) (refs 8, 9) observations of the ν_2 and ν_6 fundamental vibration bands of CH_3D in the 5- and $10\text{-}\mu\text{m}$ windows, respectively. The value of D/H in methane for Saturn is the mean of our determination ($\text{D}/\text{H} = (1.7^{+1.7}_{-0.8}) \times 10^{-5}$) and that derived from analysis of the Voyager spectra ($\text{D}/\text{H} = (2.2^{+1.8}_{-1.7}) \times 10^{-5}$ at $8.6 \mu\text{m}$ (ref. 10)). This good agreement between the ground-based and spacecraft observations and the excellent internal consistency among the results obtained by different observers using different bands, both in emission and absorption, adds to our confidence in the D/H values displayed in Fig. 1. The error bars shown there are our best estimate of the maximum ranges associated with each value and correspond to between ± 2 and ± 3 standard deviations (s.d.).

The largest reservoir of deuterium in the Solar System consists of the deuterium in gaseous hydrogen that has been preserved in an unaltered state since the formation of the solar system. The value of D/H in this reservoir should be close to 2×10^{-5} , the value deduced for the primordial solar nebula from studies of ^3He in meteorites and the solar wind¹¹⁻¹⁴. This is indeed the value deduced from the average of the measurements of D/H

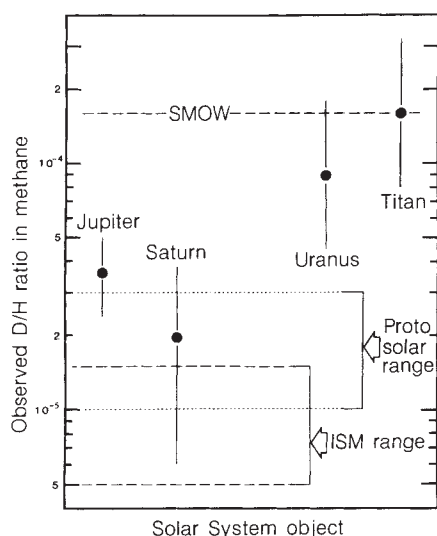
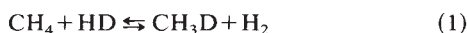


Fig. 1 Stoichiometric D/H ratios in the methane component of the atmospheres of Jupiter, Saturn, Uranus and Titan ($D/H = 1/4 [CH_3D/CH_4]$). The values for Uranus and Titan are taken from our analysis of CH_3D in their spectra at $1.6 \mu m$ (refs 1, 3, 4); Saturn's value is a mean of our value^{1,2} and that derived from the Voyager observations¹⁰; Jupiter's value is a mean of ground-based^{6,7} and Voyager^{8,9} results. See text for a discussion of the fractionation factor needed to relate these values to equilibrium D/H in the hydrogen component. For comparison, the value for Standard Mean Ocean Water (SMOW) is presented along with recent estimates for the interstellar medium²¹ (ISM), and the primitive solar nebula¹¹⁻¹⁴ (Protosolar).

in methane in the atmospheres of Jupiter and Saturn, where it is mixed with a nearly cosmic abundance of warm hydrogen gas.

To measure this value directly, a small correction must first be applied to the D/H ratio given in Fig. 1, to account for deuterium fractionation between the hydrogen and methane. Fractionation leads to a slight enrichment of deuterium in methane over that in the hydrogen reservoir according to a series of exchange reactions summarized by:



Beer and Taylor¹⁵ considered this problem in detail for Jupiter and found that the internal temperatures and rates of convection are high enough to support equilibrium in this exchange sequence. They estimated that the D/H ratio in methane should be enhanced relative to that in hydrogen by a factor of 1.37 (ref. 15). Homologous arguments lead to a similar result for Saturn. Application of this correction factor to the mean D/H ratio in methane on Jupiter and Saturn, 3.0×10^{-5} , yields a D/H ratio in gaseous hydrogen of 2.2×10^{-5} , in excellent accord with the 3He results.

Current estimates of the present-day abundances of H_2 and HD in the Jupiter and Saturn atmospheres¹⁷⁻¹⁹ are not consistent with this fractionation picture. The published D/H ratios are near 5×10^{-5} , and recent work suggests that these numbers should be closer to 10^{-4} (W. H. Smith, personal communication). Possible causes for the discrepancy may include the underestimation of the effects of scattering processes which affect the very weak (<1 mÅ) lines of HD and the presence of very weak methane absorption in the same spectral region. We favour the value for D/H in the hydrogen component of these atmospheres that is deduced from measurements of CH_3D , as this value is in good agreement with the primordial D/H ratio estimated from 3He and thus provides a more consistent picture of the reservoir of deuterium in the protosolar gaseous hydrogen. A protosolar value of D/H near 10^{-4} also seems to be incompatible with standard models of galactic evolution that must convert this ratio to the present local interstellar medium value of $(1 \pm 0.5) \times 10^{-5}$ in 4.55×10^9 yr^{20,21}.

The second, much smaller reservoir of deuterium consists of deuterium in volatiles that were trapped in or frozen onto the cold, solid materials in the protosolar nebula, without ever reaching temperatures above 450 K. This temperature is the lower limit required for the reactions represented by equation (1) to reach equilibrium in 4.55×10^9 yr. This reservoir is represented in the outer Solar System by unequilibrated gases derived from these solids. The methane in the atmosphere of Titan provides a good example. Our value of D/H in methane for Titan, $D/H = (1.65^{+1.65}_{-0.8}) \times 10^{-4}$, is in good agreement with the revised value of $\sim 2 \times 10^{-4}$ obtained by Kim and Caldwell (ref. 22 and personal communication) from an analysis of the $8.6\text{-}\mu m$ CH_3D band in Voyager spectra. (The revision is required by corrections to the temperature-pressure profile of Titan's atmosphere and new broadening coefficients for the ν_6 band of CH_3D .) The difference between D/H in methane on Titan and on Jupiter appears to be significant at the level of 2-3 s.d. The Titan value is similar to that found in the terrestrial oceans (Fig. 1) and in the hydrogen-containing compounds in meteorites¹³.

A thorough analysis of the possible processes that could enhance the D/H ratio in methane on Titan subsequent to the satellite's formation²³ suggests to us that the observed deuterium enrichment predates Titan's origin. In this analysis, Pinto *et al.* found the maximum enrichment factor to lie between 1.7 and 4.4. The high values depend on isotope exchange between methane and hydrogen promoted by a hypothetical catalysis which requires metallic grains in the proto-Saturnian nebula at a temperature near 500 K. In the absence of this unlikely process (neither the high temperatures nor the bare metallic grains are very probable in this environment), the maximum enrichment is 2.2, caused primarily by dissociation of methane and escape of hydrogen from Titan²³. It thus appears that the enhancement of Titan's D/H ratio by a factor of 8^{+8}_{-4} relative to the primordial, solar-nebula value is significantly larger than can be expected from processes acting during or after formation of the satellite.

The upper limit of 1% on the neon abundance in Titan's atmosphere set by the Voyager observations²⁴ indicates that the atmosphere is secondary, not captured²⁵. Consequently, the deuterium-enriched methane must have been outgassed from the solids, primarily the ices, in the satellite's interior. Thus, within a factor of ~ 2 , the D/H ratio in Titan's methane must correspond to the D/H ratio in the cold methane gas and methane already trapped in solid materials in the protosolar nebula from which the satellite accreted. It represents a reservoir of primordial deuterium that is distinctly different from the reservoir characterized by the methane in the atmospheres of Jupiter and Saturn. The methane on these giant planets has equilibrated with warm atmospheric hydrogen after the formation of these bodies, but evidently this equilibration never occurred during the formation of Titan. We suggest that the high enrichment found in Titan's methane was caused by ion-molecule reactions in the interstellar medium before the formation of the solar system. Such reactions proceed rapidly even at low temperatures and are known to produce even higher enrichments in some molecules^{26,27}.

This second reservoir of deuterium may also exist in the cores of all the outer planets, which similarly formed out of the rock-ice component of the protosolar nebula. Additional evidence for it may be found in the atmospheres of Uranus and Neptune, where, in contrast to Jupiter and Saturn, the outgassed deuterium-enriched methane would not be so diluted by fractionation equilibrium with the hydrogen envelope²⁸. The value of D/H in methane, $D/H = 9^{+9}_{-4.5} \times 10^{-5}$, derived for the atmosphere of Uranus, is a factor of $4.5^{+4.5}_{-2.3}$ higher than the nominal D/H ratio ascribed to the gaseous hydrogen reservoir, and the only plausible process which can explain this enhancement is the outgassing of methane from a deuterium-enriched rock-ice core. Within the uncertainties we cannot distinguish this value from that found on Titan, although our preferred values suggest that enhancement of the D/H ratio in methane may be slightly

less on Uranus. This would indeed be expected in the absence of comparable hydrogen escape, or from partial dilution through exchange with atmospheric hydrogen.

As was true for Jupiter and Saturn, the existing observations of the HD/H₂ ratio in the uranian atmosphere^{17-19,29} are too uncertain to provide an independent evaluation of D/H in the atmospheric hydrogen. It seems unlikely, however, that fractionation equilibrium (equation (1)) could have produced a measurable increase in the D/H ratio in the atmospheric hydrogen of Uranus compared with the primordial hydrogen reservoir, because the methane mixing ratio is only 0.01–0.1 (ref. 30). But a substantial enrichment of deuterium in the hydrogen component would result if much of the hydrogen itself is actually secondary, coming from the dissociation of methane. In this case the atmosphere would be deficient in helium, but might be enriched in N₂. The Voyager 2 encounter in January 1986 should test these latter consequences.

In either case, the enhancement of the D/H ratio in the atmospheric methane on Uranus is independent evidence of a second reservoir of deuterium in the protosolar nebula, which is distinct from the gaseous hydrogen reservoir and enriched by a factor of ~10. It also supports core-accretion models for outer planet formation. In their comprehensive review of deuterium enrichment in the Solar System, Geiss and Reeves¹³ found that the available evidence was inadequate to determine whether the observed enrichments occurred before or after Solar System formation. Our new data strongly support enrichment in the interstellar medium before formation of the solar nebula. A further test of this conclusion will come from the observation of deuterium in comets. Based on the scenario outlined here, we predict D/H ratios near 10⁻⁴ in the major hydrogen-bearing volatiles subliming from a cometary nucleus, values that are

2.5 × 10⁻² lower than the current upper limit reported by A'Hearn *et al.*³¹ for OD/OH. Observations of comet Halley now underway both from the ground and from spacecraft may provide this test.

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Direct laboratory determination of the ¹⁸⁷Re half-life

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The long-lived, naturally occurring radionuclide ¹⁸⁷Re is important in geochemistry and cosmology as a nucleochronometer. Until now there have been no direct laboratory measurements which have avoided the difficulties of both low-energy β-counting and dependence on radiometric ages of rocks and meteorites. We report here a half-life of (4.35 ± 0.13) × 10¹⁰ yr, based on the growth of ¹⁸⁷Os over a 4-yr period into a large source of osmium-free rhenium. Since our result agrees with the best geochemically determined values, no significant revision of the present galactic age limits based on the geochemical values is necessary. The agreement at the 5% level of the decay rates determined on laboratory and meteorite-age timescales places a tight constraint on cosmological models in which fundamental 'constants' are allowed to vary. In particular, the fine-structure constant, α, must have changed by less than one part in 10⁵ over the past 4.5 × 10⁹ yr.

Geochemists¹ and astrophysicists² have recognized that the isobaric pair ¹⁸⁷Re–¹⁸⁷Os could provide terrestrial, Solar System and cosmic chronologies. For these, the ¹⁸⁷Re half-life is a necessary parameter. Recent determinations, which indicate a probable value of ~4 × 10¹⁰ yr, rely on accepted ages for meteorites and rocks. Analysis of molybdenites of known age

by Hirt *et al.*³ yielded an average of (4.3 ± 0.5) × 10¹⁰ yr. With similar techniques applied to meteorites, Luck and Allègre⁴ deduced a half-life of (4.56 ± 0.12) × 10¹⁰ yr. Naldrett⁵ determined a half-life of (3.5 ± 0.4) × 10¹⁰ yr by counting the β-particles emitted from a known quantity of a pure rhenium compound dissolved in a liquid scintillator. His value is significantly lower than the geological³ and meteoritic⁴ values. The results from these different experiments need not agree, since the counting experiment does not detect decays to the bound states of the daughter ¹⁸⁷Os. However, the half-life from the counting experiment should be larger, rather than smaller, than that determined from rocks or meteorites.

Our investigation determined the sum of the β-decays of ¹⁸⁷Re to the bound and unbound states of ¹⁸⁷Os by measuring the growth of ¹⁸⁷Os atoms in a series of 100-g sources of osmium-free perrhenic acid (HReO₄) solution. Each source differed only in the time interval allowed before osmium removal and measurement. Traces of residual osmium were first removed from 1 kg of concentrated perrhenic acid by exhaustive reflux-distillation. Thus the initial ¹⁸⁷Os/HReO₄ ratio of 5 × 10⁻⁸ was reduced to 7 × 10⁻¹².

The rhenium was 'spiked' with independent gravimetrically prepared solutions of isotopically enriched ¹⁹⁰Os (98%) and ¹⁹²Os (99%). The rhenium solution was then frozen at –65 °C for about 2 yr, thawed at –35 °C just long enough to remove a 10% aliquot, and refrozen. Osmium was then distilled from the aliquot, purified, and concentrated for isotopic analysis. The thawing and sampling cycle was repeated on the perrhenic acid three times over a 1.5-yr interval following the first sampling. Thus, a single kilogram of rhenium provided a series of ¹⁸⁷Os growth points relative to the two independent 'spikes'. Since the ¹⁸⁷Re/¹⁹⁰Os and ¹⁸⁷Re/¹⁹²Os ratios were fixed by the spike additions at the onset of growth, we needed only to determine the rate of change of the ¹⁸⁷Os/¹⁹⁰Os and ¹⁸⁷Os/¹⁹²Os ratios in the rhenium solution from the isotopic analyses of the osmium samples.

Table 1 Osmium isotope ratios from perrhenic acid source*

Sample no.	Growth time (days)	$^{187}\text{Os}/^{190}\text{Os}$		$^{187}\text{Os}/^{192}\text{Os}$	
		LAMMA†	ICP-MS‡	LAMMA†	ICP-MS‡
1	670	1.360 ± 0.018	1.359 ± 0.013	1.418 ± 0.019	1.428 ± 0.014
2§	691	1.387 ± 0.022	1.405 ± 0.011	1.445 ± 0.021	1.481 ± 0.016
3	883	1.672 ± 0.021	1.682 ± 0.015	1.750 ± 0.022	1.761 ± 0.016
4	1,236	2.177 ± 0.054	2.178 ± 0.027	2.258 ± 0.047	2.290 ± 0.034

* Uncertainties expressed as 1 s.d.

† Corrected for isobaric interferences and estimated mass bias, with no uncertainty added. Additional systematic uncertainties of ±1.5% for $^{187}/^{190}$ and ±1.8% for $^{187}/^{192}$ should be assigned for these corrections.

‡ Corrected for mass bias and for contamination with natural osmium. Uncertainties in these corrections have been included.

§ Correction of (2.50 ± 0.25)% applied for ^{187}Os content of radioactive ^{185}Os tracer added in this sample.

In order to monitor chemical yields in one of our samples, we added ^{185}Os radioactive tracer, made by bombarding tungsten with 65-MeV α -particles. Since the bombardment produced a spectrum of stable osmium isotopes, which we measured independently⁶, a small correction was made for the ^{187}Os introduced into the sample with the ^{185}Os (see Table 1).

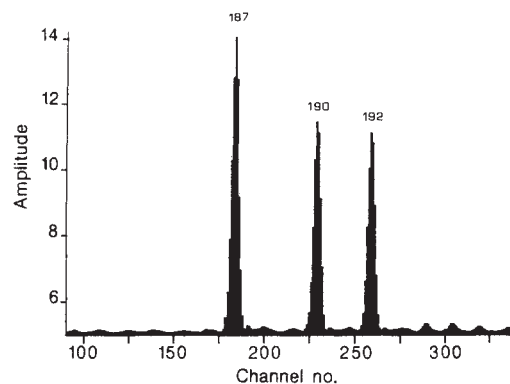
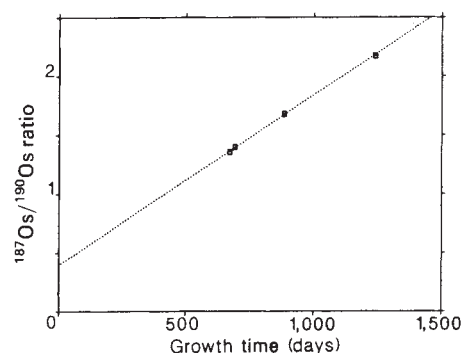
Osmium isotopic analyses were performed with two different types of mass spectrometer: a laser microprobe mass analyser (LAMMA), which combines a pulsed-laser ablation ion source with a time-of-flight mass spectrometer⁷, and an inductively coupled plasma mass spectrometer (ICP-MS), which uses an RF-excited argon plasma torch as the ion source for a quadrupole mass spectrometer⁸.

The LAMMA has high sensitivity but it has two limitations⁹. The first is a low threshold for saturation effects in the detector, the second the imprecision of the waveform digitizer in the detector system. These effects limit the ultimate precision of LAMMA isotope ratios. However, we were able to obtain mass abundance ratios with a standard deviation of ~1% by accumulating large numbers (~100) of individual spectra, performing channel-by-channel summation of all spectra and taking ratios of the resulting summed peak areas. Corrections for molecular-ion contributions and uncertain mass-bias effects¹⁰ increase the uncertainty by another estimated 1–2%.

Figure 1 shows the summation of 100 selected LAMMA spectra of the osmium sample isolated from the first 100 g of rhenium. Apart from small amounts of platinum (masses 194 to 196), and an even lower level of molecular-ion contaminants, the three expected osmium peaks stand out clearly. Significantly, the 'signal' at mass 185 is the smallest of any shown in Fig. 1. Interpreted as ^{185}Re , this implies that no more than a few parts in 10^{13} of the parent rhenium aliquot were carried into the final osmium sample.

In contrast, the ICP-MS was somewhat less sensitive than LAMMA, even with the osmium response strongly enhanced by introduction of the osmium as the tetroxide vapour into the argon torch¹¹. However, the combination of ion counting and mass scanning over a relatively stable osmium ion beam, essentially free of isobaric interferences, resulted in mass spectra of better quality than those from LAMMA. Mass fractionation was substantial (~1.8% per mass unit), but it was determined with adequate precision by bracketing each run with natural osmium isotopic standards. For the isotopic abundances of natural osmium, we used the values reported by Nier¹².

Isotope ratio data from both techniques are given in Table 1. Although two of the four ICP-MS measurements showed instrumental contamination with natural osmium, we used a two-

**Fig. 1** Mass spectrum of osmium region; LAMMA data. The osmium sample was isolated from 100 g of perrhenic acid after a growth period of nearly 2 yr.**Fig. 2** Time dependence of the $^{187}\text{Os}/^{190}\text{Os}$ ratio; ICP-MS data. The dotted line is the least-squares fit.

component analysis to determine the experimental osmium composition. Figure 2 shows the time dependence of the isotope ratio $^{187}\text{Os}/^{190}\text{Os}$ for the ICP-MS data. The dotted line is the least-squares fit. The non-zero intercept indicates that ~8 ng of ^{187}Os had not been removed before the onset of growth. The slope of the least-squares-fit line in Fig. 2 determines the half-life from the expression

$$t_{1/2} = \left(\frac{^{187}\text{Re}}{^{190}\text{Os}} \right) \frac{\ln 2}{\text{line slope}}$$

in which ^{187}Re and ^{190}Os are derived from the gravimetric standardizations of the solutions. Similarly, independent half-life determinations on LAMMA and ICP-MS were made from the ^{192}Os spike. These results are summarized in Table 2.

We find a 3–4% systematic difference between the measured ($^{190}\text{Os}/^{192}\text{Os}$) ratios and the values calculated from the

Table 2 ^{187}Re half-life values (10^{10} yr)

Method	^{190}Os spike	^{192}Os spike
LAMMA	4.27 ± 0.23	4.44 ± 0.24
ICP-MS	4.29 ± 0.19	4.41 ± 0.21

The weighted average value is $(4.35 \pm 0.13) \times 10^{10}$ yr.

gravimetric data for the standard solutions of ^{190}Os and ^{192}Os . However, X-ray fluorescence analyses of both solutions confirm the absolute values of the gravimetric determinations within the current 5% uncertainty of the X-ray method (F. Bazan, personal communication).

Our half-life value agrees with that of Hirt *et al.*³ and is only slightly lower than the determination of Luck and Allègre⁴. The

agreement at the $(5 \pm 6)\%$ level (estimated 95% confidence interval) of the meteoritic half-life value and our laboratory determination can be used to place limits on the possible variation of the fine-structure constant α , predicted by some cosmological models¹³. Following Dyson¹⁴, we calculate the average rate of change over the past 4.55×10^9 yr to be

$$\left\langle \frac{\dot{\alpha}}{\alpha} \right\rangle = (-1 \pm 2) \times 10^{-15} \text{ yr}^{-1}$$

Due to the small decay energy of ^{187}Re , these are the tightest limits on the possible variation of α currently allowed by analysis of β -decay data. However, much tighter limits ($|\dot{\alpha}/\alpha| < 10^{-17} \text{ yr}^{-1}$ (ref. 15) and $|\dot{\alpha}/\alpha| < 2 \times 10^{-18} \text{ yr}^{-1}$ (ref. 16)) over the past 2×10^9 yr have been claimed from analysis of ^{149}Sm neutron-capture depletion in the Oklo natural reactor.

With regard to the question of the age of the elements, our half-life value is so close to the one assumed by Clayton² that his proposed age limits for galactic nucleosynthesis ($11\text{--}18 \times 10^9$ yr) are still valid.

We thank Dr Barry Berman for pointing out the need for an accurate half-life value for ^{187}Re in geochemistry and cosmology, and for many discussions in the early stages of this work. We also thank Professor D. D. Clayton for encouragement, Dr G. B. Hudson for discussions on cosmological implications, and

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Evidence of changing concentrations of atmospheric CO_2 , N_2O and CH_4 from air bubbles in Antarctic ice

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Atmospheric carbon dioxide (CO_2) levels before the industrial revolution were $\sim 260\text{--}280$ p.p.m.v. (parts per 10^6 by volume) as determined from studies of air trapped in ice^{1,2}. We report here similar results, using Antarctic ice, for the CO_2 levels during the seventeenth and eighteenth centuries, which suggest an average concentration of 281 (standard deviation $\sigma = 7$) p.p.m.v. The data constrain the net release of biospheric carbon to the atmosphere over the past 200 yr, to $\sim 5 \times 10^{10}$ tonnes of carbon, mostly during 1850–1900. Measurements of two other 'greenhouse' gases, methane (CH_4) and nitrous oxide (N_2O), show increases of about 90 and 8% respectively since 1600. This CH_4 increase is similar to the recently reported^{3–6} doubling over the same period, and the N_2O increase, the first direct evidence of historical changes in N_2O , is consistent with releases due to expanding anthropogenic combustion processes⁷.

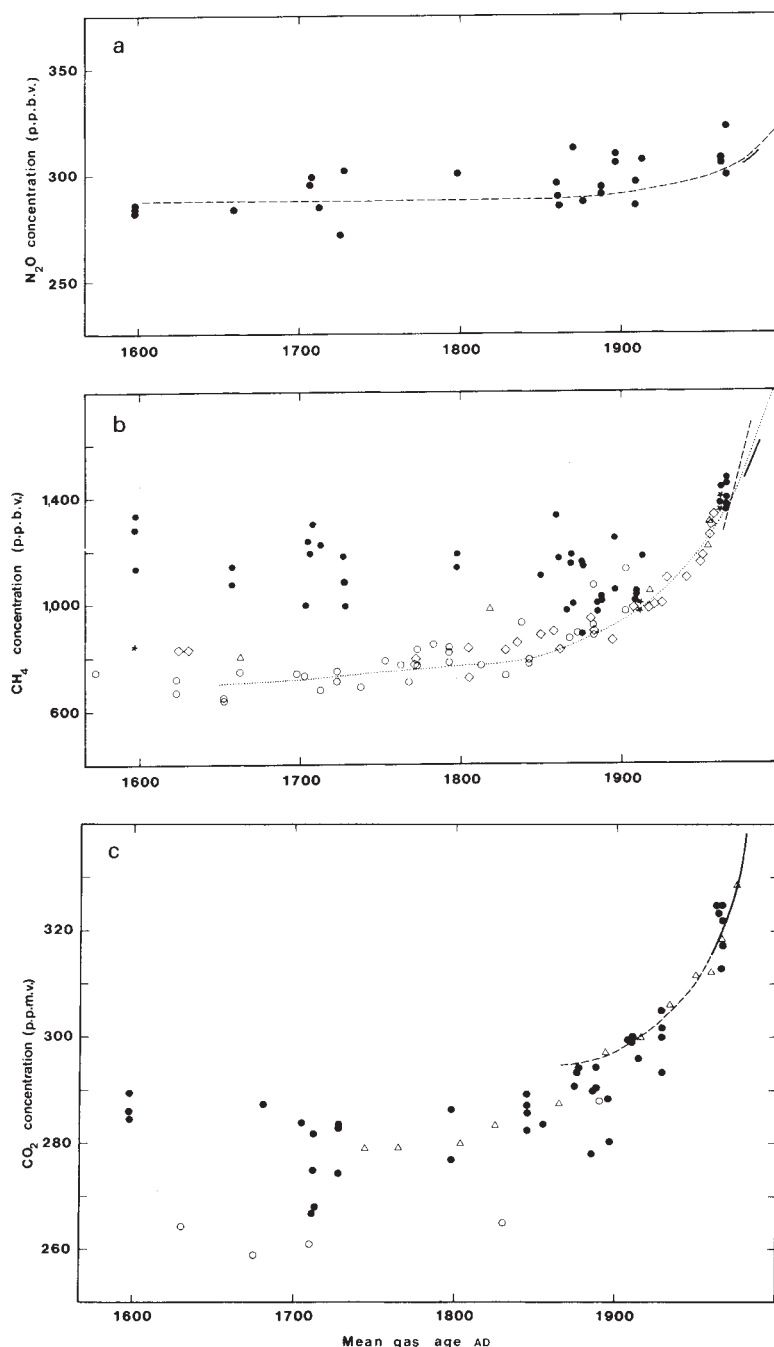
The ice core (BHD) used was thermally drilled to a depth of 473 m near the summit of Law Dome, Antarctica ($66^\circ 43' \text{S}$, $112^\circ 50' \text{E}$; 1,375 m) by the Australian National Antarctic Research Expedition in 1977. High precipitation rates (0.65 m yr^{-1}), lack of melting at the firn surface (annual average temperature $= -22^\circ \text{C}$), a simple flow regime and small snow dunes all combine to make Law Dome an ideal location for ice-gas composition studies. Although accurate ice dating (± 2 yr at 1912, ± 6 yr at 1850) has been achieved using oxygen isotope stratigraphy⁸, the age of the ice is not the same as that of the occluded air bubbles. However, using recently reported relationships between date of occlusion and ice density for a similar site⁹, the ice is deduced to be 67 yr older than the enclosed air

with 80% of the gas being trapped over the relatively short period of 13 yr.

A complete description of gas extraction and analysis methods used in this study will be given elsewhere. Briefly, a dry extraction technique was developed to crush defect-free and trimmed (0.5–5 cm) core samples (0.5–1.4 kg) to fine powder in ~ 4 min at -80°C , releasing approximately 90 cm^3 of air per kg at STP. The released air was dried (-100°C) and condensed into stainless steel traps at liquid helium temperature. The latter were warmed and agitated for 24 h to ensure uniform mixing. Gas chromatography was used to determine CO_2 and CH_4 concentrations to precisions of 0.5 p.p.m.v. and 10 p.p.b.v. (parts per 10^9 by volume) respectively using flame-ionization detection, and N_2O to 2 p.p.b.v. using electron-capture detection. To demonstrate whether the technique affected trace gas concentrations in air released from ice, air of known composition was exposed to freshly crushed, evacuated ice, and then re-analysed. CO_2 and N_2O concentrations were retrieved to within 0.5%, with no bias, while returned CH_4 concentrations averaged 20 p.p.b.v. or 1.5% high. The CH_4 data reported here are corrected for this effect. A similar test, involving the crushing of small ice samples (15 g) in the presence of air of known composition, showed similar results for CO_2 and N_2O , but substantial CH_4 enhancement. The data represent averages of 1–4 chromatographic analyses.

A total of 75 core samples were crushed and analysed, although due to the availability of the equipment, not all three gases were measured on all samples. There were 44, 69 and 74 samples measured for N_2O , CH_4 and CO_2 respectively. On examination of the data, it was found that sections of the core, exhibiting visible evidence of post-coring melting (PCM), yielded concentrations that were variable and significantly lower than average for CO_2 and N_2O and higher for CH_4 . For N_2O , samples exhibiting severe signs of PCM were rejected, leaving 27, with a further sample removed because its concentration was 100 p.p.b.v. less than the average of all the remaining selected data (average 295; $\sigma = 12$ p.p.b.v.). For CH_4 , 44 samples survived the PCM examination; two of the remaining samples yielded CH_4 concentrations 450 and 570 p.p.b.v. higher than the average pre-1920 data (1,115; $\sigma = 116$ p.p.m.v.), and were therefore rejected. While extremely low CO_2 concentrations were related to extremes of PCM, it appeared that CO_2 variability

Fig. 1 Historical record of atmospheric N_2O , CH_4 and CO_2 concentrations based on measurements of air trapped in the BHD ice core from Law Dome, Antarctica (●). *a*, Solid line, modern data for the South Pole, 1976–79 (ref. 7), and from Cape Grim, Tasmania, 1978–84 (ref. 14). Dashed line, a global tropospheric average concentration for a model incorporating constant non-combustion and growing combustion sources of N_2O (ref. 7). Model and South Pole data reported by Weiss⁷ are increased by 2% to agree with Cape Grim data. N_2O data are relative to secondary standards¹⁵ provided by R. A. Rasmussen, multiplied by 0.92 to convert to absolute concentration. *b*, Measurements made after elimination of CH_4 enhancement due to metal-metal friction (★). The other data (●) are believed to require correction downwards by ~400 p.p.b.v. in the fifteenth to sixteenth centuries, the correction decreasing to negligible amounts for modern samples (see text). Modern data are from Cape Grim, 1978–84 (solid line) (refs 16, 17), and from the Northern Hemisphere, 1965–81 (dashed line) (ref. 18). Data from other studies (Δ , ref. 4; $\circ$, ref. 5; and $\diamond$, ref. 6) are included for comparison. Dotted line, a global tropospheric average concentration from a model incorporating constant natural and growing anthropogenic sources of CH_4 , and decreasing levels of atmospheric OH (ref. 11). *c*, Solid line, modern data for the Mauna Loa Observatory¹⁹. Data from other ice studies (Δ , ref. 1; $\circ$, ref. 2) are included for comparison. Dashed line, back-extrapolation from the observed concentration in 1959 assuming a constant airborne fraction of 0.57 (ref. 20), using the fossil fuel release data of Rotty²¹. CO_2 data are reported in the World Meteorological Organization 1981 CO_2 calibration scale.



was associated with subtle and less easily quantified evidence of PCM. Thus for CO_2 , a datum was selected if its concentration was within 7 p.p.m.v. of at least one other sample of comparable age (± 5 yr). This criterion resulted in the selection of 42 samples, 30 of which showed little or no evidence of PCM. The remaining 12 had more than 2 cm of outer ice removed before crushing and this may have explained their apparent independence of PCM.

The selected N_2O data are shown in Fig. 1*a*. The ice data for recent times agree with modern measurements and a linear regression of the whole record shows that concentrations have increased by 6 ($\sigma = 2$) p.p.b.v. per 100 yr over the past 300 yr. This is the first evidence of changing atmospheric N_2O levels on this timescale. The current trend of 0.5–1.8 p.p.b.v. yr^{-1} (refs 7, 10) is not characteristic of the 400-yr ice record. N_2O averaged 289 ($\sigma = 10$) p.p.b.v. between 1600 and 1800 compared with modern Southern Hemisphere values of about 310 p.p.b.v. A similar pre-industrial concentration was deduced by Weiss⁷

(Fig. 1*a*) using a combustion source model to fit modern N_2O concentrations and their rate of change with time.

The selected CH_4 data are shown in Fig. 1*b*, together with results from other ice studies. Again, there is reasonable agreement between modern measurements and the recent ice core records. The new data suggest that atmospheric CH_4 levels over the period 1600–1900 averaged 1,115 ($\sigma = 116$) p.p.b.v., 40% lower than current concentrations, with the most significant changes occurring during this century. The results are in contrast to other studies which showed that CH_4 concentrations have doubled since the seventeenth century or earlier^{4–6}, with a rapid rise starting in the early nineteenth century. This discrepancy led us to investigate and demonstrate the production of CH_4 in our apparatus by frictional contact between metal surfaces, as has been observed elsewhere⁶. Subsequent analyses of a further five core samples (see Fig. 1*b*), with this friction eliminated during ice crushing, show good agreement with other studies. These limited results suggest that CH_4 production is greater with

old ice than with more modern ice. The calibration tests involving ice crushing showed that for gas samples of low CH₄ concentration (~700 p.p.b.v.), the contamination was significant (~400 p.p.b.v.). At higher concentrations (1,800 p.p.b.v.) the CH₄ enhancement was significantly less. Thus, metal-to-metal friction in our apparatus has enhanced CH₄ concentrations observed in ice-cores containing fifteenth to sixteenth century air by ~400 p.p.b.v., while the effect on modern air samples has been negligible. Correcting for this effect would bring our CH₄ data into approximate agreement with previous studies, but the variability in our data caused by variations in the amount of CH₄ produced by friction cannot be allowed for. Improvements to the crushing device have since eliminated the problem.

The selected CO₂ data are presented in Fig. 1c, together with the Swiss¹ and French² data. As with N₂O and CH₄, the agreement between modern atmospheric measurements and air trapped recently in ice is encouraging. The data indicate CO₂ levels of about 290–295 p.p.m.v. during the last two decades of the nineteenth century, which are supported by chemical measurements made at that time¹². As with the Swiss data the results suggest that mid-nineteenth-century and earlier concentrations were lower, averaging 281 ± 7 p.p.m.v. for the period 1600–1800, and 288 ± 5 p.p.m.v. for the nineteenth century. This suggests that a rapid increase in atmospheric CO₂ of about 13 p.p.m.v. (294–281) occurred during the second half of the nineteenth century or early twentieth century, that cannot be explained by the small amount of fossil fuel consumed during this period. Assuming an airborne fraction of 0.57 this indicates a net CO₂ source, presumably largely biospheric, of 4.8 × 10¹⁰ tonnes. More precise estimates of this source will be possible using a global carbon-cycle model¹³, when the concentration history is better defined.

We are confident that other sections of this core, when transported to Australia in more controlled conditions, will yield additional, better quality data on these and other gaseous constituents. Such data are invaluable in understanding the biogeochemical cycles and abundance of these species in the atmosphere, and assessing their potential role in past climate change.

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Palaeotemperatures still exist in the Greenland ice sheet

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The temperature distribution through the Greenland ice sheet at the Dye 3 borehole is a record of the past climatic changes in the Arctic. The numerical model of the temperature distribution now presented reproduces the observed temperature distribution within 0.03 K, and shows that the basal ice is still cooled 5 K by the cold ice-age climate. The results suggest a mean ice-age temperature of $-32 \pm 2^\circ\text{C}$, which is 12 K colder than the present temperature, and a precipitation rate $50 \pm 25\%$ of the present rate. Calculations of a more detailed temperature history through the present interglacial period reveal evidence of the AD 1920–50 maximum, the little ice age, and the Atlantic period.

The temperature distribution (Fig. 1a) in the 2,037-m deep, liquid-filled borehole at Dye 3 (65° N, 44° W) was measured in 1980, in 1982 and twice in 1983¹. The 1982 and 1983 temperatures were identical, within the calibration accuracy (0.03 K) of the thermistors used. The disturbances induced by the core drilling in 1979–81 have obviously disappeared, and the 1983 temperature profile therefore represents the steady-state hole temperature. The hole temperature reproduces the undisturbed ice temperatures; deviations caused by small convection cells¹ in the liquid-filled hole are small and are neglected in this work. The bottom temperature is -13.22°C , so no melting occurs at the base.

Dye 3 is situated 40 km east of the local ice divide of the ice sheet. The flow leading to the borehole is reasonably well known from surface elevations^{2,3}, strain rates⁴, surface velocities⁵, precipitation rates and model calculations^{6–8}. In outline, the ice flow follows the local ice divide northward from South Dome (64° N, 45° W) and turns at an angle of 60° to the ice divide towards the Dye 3 site. The surface velocity at Dye 3 is as high as 13 m yr⁻¹ and any temperature model must therefore take into account horizontal advection and internal deformation heating.

The model presented here considers 2,000 m of ice on top of 3,000 m of rock. The heat transfer equation for non-steady state may be written:

$$\frac{\partial T}{\partial t} = k \nabla^2 T - v \nabla T + \frac{f}{\rho c} + \left(\frac{\partial k}{\partial T} + \frac{k}{c} \frac{dc}{dT} \right) \left(\frac{\partial T}{\partial z} \right)^2 \quad (1)$$

where T is the temperature, t the time, z the depth, v the ice velocity, ρ the ice density and f the internal deformation heating. k and c , the thermal diffusivity and specific heat capacity of ice, are treated as in ref. 9.

Solutions to equation (1) are calculated numerically with the following assumptions: (1) The problem is assumed to be two-dimensional (flow direction, x , versus depth, z). (2) The ice thickness and the surface lapse rate have remained constant in time. (3) The velocity profiles are adequately represented by the Dansgaard-Johnsen model¹⁰ with a 300-m-thick bottom shear-layer, which approximates the velocity profiles revealed from measurements of the tilting of the Dye 3 borehole¹. The velocity profiles are assumed to change with time in such a way that the ice sheet maintains mass balance. (4) The horizontal temperature gradient $\partial T / \partial x$ is assumed to be proportional to the thinning of the annual layers with depth¹¹, and the horizontal heat conduction $k \partial^2 T / \partial x^2$ is negligible. (5) The geothermal heat flux is constant at the base of the 3,000 m of rock, at a value typical for Precambrian shield.

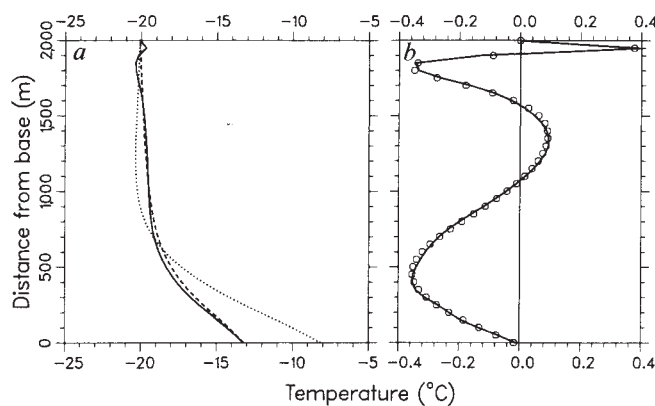


Fig. 1 *a*, Solid line, measured temperature distribution from the Dye 3 borehole. Dotted line, steady-state temperature distribution calculated for present climatic conditions. Dashed line, calculated non-steady-state temperature distribution. The smoothed climate history used in this calculation is as shown in Fig. 2 with $(T_{ice}, \lambda_{ice}, Q_{geo})$ values $(-32^\circ\text{C}, 0.245\text{ m yr}^{-1}, 38.6\text{ mW m}^{-2})$. *b*, The deviations between the measured (solid line) and the calculated (dashed line) temperature distributions from *a* are shown by the solid line. The deviations represent the more detailed temperature history of the present interglacial period and the circles show the calculated fit to these deviations using the temperature variation on Fig. 5 (solid line). The deviations between this fit and the measurements are $<0.03\text{ K}$.

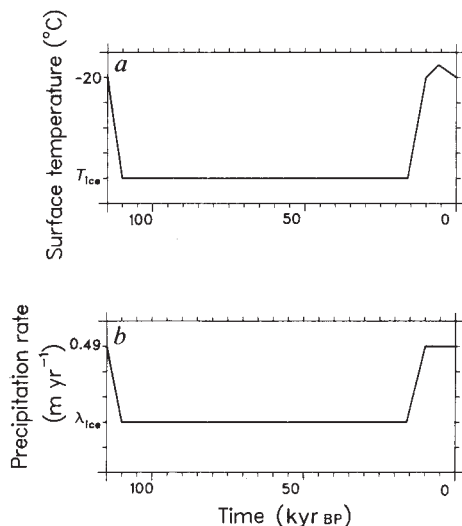


Fig. 2 The smoothed climate history used to calculate the temperature distribution shown on Fig. 1*a*. *a*, The temperature variation through the 115-kyr glacial cycle. T_{ice} is the ice-age temperature. *b*, The precipitation rate variation through the cycle. λ_{ice} is the ice-age precipitation.

An initial steady-state temperature profile was calculated for present conditions; that is, using the present surface temperature and precipitation rates. The profile deviates considerably from the observed (Fig. 1*a*). This is seen most clearly at the base, where the steady-state temperature is 5 K too high, indicating that the temperature distribution in the borehole is far from a steady-state distribution. The colder bottom temperature is a remnant from the cold ice-age climate.

A non-steady-state solution can now be calculated by applying a simplified climate history (that is, surface temperature and precipitation rate) as input to the model. The climate history throughout the last glacial cycle, the past 115 kyr BP, is repeated 7–10 times, until the initial state is ‘forgotten’ and the tem-

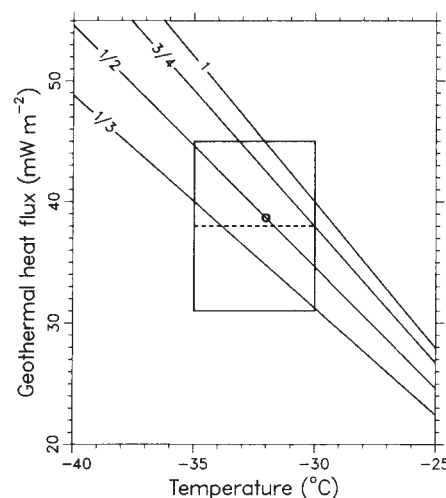


Fig. 3 The curves represent the parameter combinations $(T_{ice}, \lambda_{ice}, Q_{geo})$ which give temperature distributions similar to the observed one. The ice-age precipitation rate (λ_{ice}) relative to the present (0.49 m yr^{-1}) is shown. The circle is the parameter choice used in the further calculations. $(T_{ice}, \lambda_{ice}, Q_{geo}) = (-32^\circ\text{C}, 0.245\text{ m yr}^{-1}, 38.6\text{ mW m}^{-2})$.

perature profile responds periodically to the periodic forcing.

Figure 2 shows a smoothed version of the 115-kyr-long glacial cycle. The mean ice-age temperature, T_{ice} , and the mean ice-age precipitation rate, λ_{ice} , are considered as unknown. Solutions are calculated for a variety of (T_{ice}, λ_{ice}) values. In each case, the geothermal heat flux Q_{geo} is adjusted so as to make the resulting temperature profiles fit the observed temperature distribution (Fig. 1*a*). The results are presented on Fig. 3. Obviously, a wide span of (T_{ice}, λ_{ice}) values might represent reasonable parameter choices, because the geothermal heat flux in Greenland is poorly known. However, the mean geothermal heat flux for Precambrian shield is 38.7 mW m^{-2} (ref. 12), and this is adopted below.

As for T_{ice} , the mean ice-age temperature at Dye 3 is believed to lie in the range from -35 to -30°C . The upper limit -30°C represents a cooling of 10 K from the present surface temperature. This must be a minimum because the present cooling of the bottom ice is 5 K. The $\delta^{18}\text{O}$ profile along the Dye 3 deep core¹³ has a 7‰ shift at the Pleistocene/Holocene transition. Many uncertainties are connected with the conversion of this δ -shift to a temperature shift, but it is believed to correspond to no less than 10 K. The CLIMAP reconstruction¹⁴ of the Last Glacial Maximum (LGM) climate has a maximum sea-surface temperature anomaly in the North Atlantic. This anomaly between modern and LGM conditions is 14 K, and the anomaly at Dye 3 is not believed to exceed this maximum, so the lower limit of the mean ice-age temperature is probably -35°C .

Figure 3 shows that the curves with a mean precipitation rate, λ_{ice} , 33–75% of the present are the most realistic. This conclusion agrees with the measurements of the radioisotope ^{10}Be in the deep core¹⁵.

The calculated variation of the temperature profile throughout the glacial cycle is shown in Fig. 4. The cold glacial climate cooled the surface from 115 kyr BP, but the warming of the basal ice continues to 110 kyr BP. It was only at 65 kyr BP, that is, 50 kyr after the onset of the glaciation, that the basal temperature reached a minimum and the temperature profile got close to a steady-state solution for glacial conditions. The surface temperature variation of 12 K is damped to 5 K at the base. The variation of the temperature distribution, and the time (50 kyr) required to reach a steady-state profile clearly demonstrates the necessity of using a non-steady-state model.

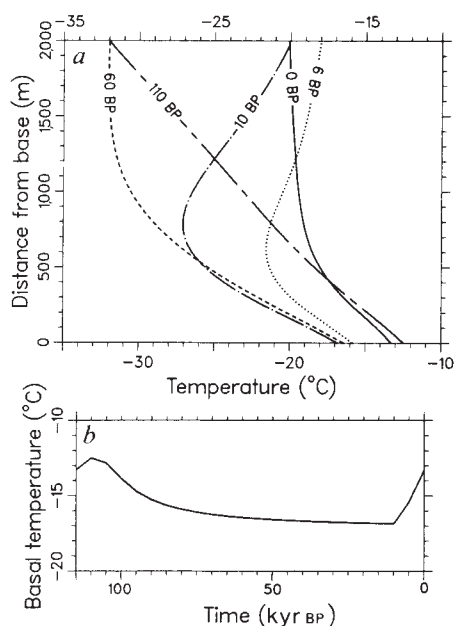


Fig. 4 *a*, Variation of the temperature distribution through the 115-kyr glacial cycle shown on Fig. 2. *b*, Variation of the basal temperature through the glacial cycle.

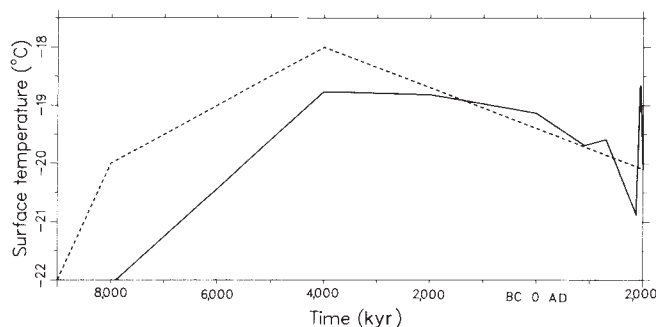


Fig. 5 Solid line, the more detailed temperature variation through the past 10 kyr BP, derived from the observed temperature distribution at the Dye 3 bore-hole. Dashed line, smoothed temperature history also shown on Fig. 2a.

The deviations between the smoothed and the observed temperature profiles are shown on Fig. 1b. These deviations are used to calculate a more detailed atmospheric temperature history through the past 10 kyr, the present interglacial period. A least-squares method is used, and the detailed temperature variation is added to the last 115-kyr glacial cycle, used as an input to the non-steady-state model. The temperature trend curve in Fig. 5 results in a calculated temperature profile along the deep hole which deviates <0.03 K from the measured profile (Fig. 1b). A study shows that the calculations through the past 10 kyr are rather independent of the 'ice-age solution', that is, the choice of (T_{ice} , λ_{ice}) used to calculate the smoothed temperature profile along the deep core.

The calculated temperature record in Fig. 5, particularly through the recent millennia, agrees well with what is known of post-glacial temperature trends in the Arctic troposphere^{16,17}. The AD 1920–50 maximum, the little ice-age (AD 1400–1900), the medieval warm period (AD 900–1400); the cold period in early medieval times, and the warm Roman time are recorded in most of the Northern Hemisphere. The onset of the warm Atlantic period is also revealed at 4000 BC, but its termination around 1000 BC is missing. However, note that the time resolu-

tion and the accuracy of the climate history revealed by the Dye 3 deep hole decrease backward in time, because the temperature variations in the borehole are gradually smoothed with increasing depth in the ice sheet.

The numerical modelling shows that the temperature distribution in the ice sheet contains considerable information on past climatic conditions. The information is most detailed in the past 10 kyr, because the resolution in the ice is better and the ice flow and precipitation were similar to the present well-known conditions. The glacial climate can be estimated by the model. The results in Fig. 3 demonstrate that a range of solutions are acceptable. This fact, combined with the unknown ice thickness and ice flow¹⁸ in glacial conditions, limits the resolution of the ice-age climate.

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Geophysical modelling of the thermal history of foreland basins

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Rapid subsidence and sedimentation in active foreland basins produces negative thermal anomalies within the sediments and the lithosphere. We have developed a one-dimensional conductive heating model to simulate the thermal effects of subsidence in foreland basins. Application of the model to data from a well in the Alberta Basin suggests that negative thermal anomalies can delay hydrocarbon maturation by a few million years. The thermal anomaly is larger within the lithosphere than in the sediments. As the anomaly is removed by conductive heating the rheological properties of the lithosphere are modified, causing an apparent reduction of plate rigidity. The thermal anomaly in the lithosphere also results in a positive buoyancy force which acts against the mechanical loading of the plate and produces thermally driven uplift when active loading ceases.

We have modelled the subsidence of foreland basins as the response of an elastic or a viscoelastic plate to loading during orogenic compression^{1–3}. A relationship has been shown to exist between the thermal state of the continental lithosphere and its

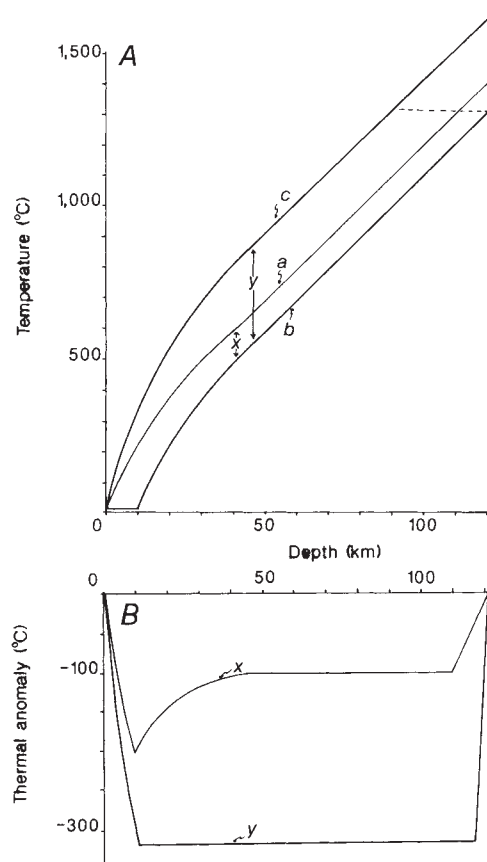


Fig. 1 The thermal anomaly resulting from a single 10-km episode of subsidence of continental lithosphere. **A**, The original, post-instantaneous-subsidence and final equilibrium thermal gradients are *a*, *b* and *c*, respectively. The difference between the initial and final equilibrium gradients (*y* minus *x*) is the result of thermal blanketing of the added sediments. **B**, The thermal anomalies are *x*, the anomaly resulting from depression of the slab, and *y*, the anomaly resulting from both the depression of the slab and the thermal blanketing effect of the sediments.

response to mechanical loading³⁻⁵. Rapid deposition of cold, low conductivity sediments results in the formation of negative thermal anomalies (the difference between actual temperatures and equilibrium temperatures) within the sediments and underlying lithosphere. As a result of thermal blanketing, the sediments will equilibrate with a new, generally hotter, equilibrium geothermal gradient, which adds to the negative anomaly (Fig. 1). In our model, the temperature of the asthenosphere during deposition of each increment of sediment is set equal to the equilibrium temperature resulting from the deposition of that increment of sediment. Thus, a sharp temperature change occurs at the interface between the lithosphere, which is not in thermal equilibrium, and the underlying asthenosphere (Fig. 1).

In foreland basins of western North America (Fig. 2), the subsidence resulting from thrusting in the Cordilleran orogenic belt occurs in multiple, rapid episodes with sedimentation rates sufficient to keep the basins nearly filled⁶. This mechanically driven subsidence began between 200 and 160 Myr ago and ended ~50 Myr ago, and was synchronous with thrusting in the adjacent Cordillera. The magnitude of subsidence decreases with distance from the mountain belt, as is shown by the subsidence curves (Fig. 2). Each episode of subsidence, presumably resulting from a distinct phase of thrusting^{1,2}, produces negative thermal anomalies. Because heat conduction is slow relative to the frequency and magnitude of the subsidence episodes, a portion of the thermal anomalies resulting from each subsidence episode will be present at the initiation of the next episode.

Thus, non-equilibrium conditions form the initial conditions for all thermal calculations after foreland basin subsidence begins. The thermal evolution is calculated using a one-dimensional finite difference method modified from ref. 7, and we assume that all heat transfer is conductive. A one-dimensional calculation produces an acceptable approximation of the thermal history because foreland basins tend to be broad features in which lateral temperature gradients are likely to be small.

The record of subsidence preserved in the stratigraphy of the foreland basin forms the primary input for our thermal model. Sediment compaction is estimated by the method of Bond and Kominz⁸ which was modified to include present-day porosity in the basin strata. Density, specific heat and concentration of radioactive material are calculated as the arithmetic mean of these properties for the appropriate mix of various sediment types and water⁹. We assume that porosity varies with both lithology and depth⁸. Sediment conductivity is calculated according to the Maxwell diffusion equation¹⁰ treating the sediments as a dispersed phase in water. The thermal model is independent of uncertainties in parameters such as palaeo-water depth (or height above sea level), palaeo-sea level (the definition of a fixed base level) and the flexural response of the lithosphere-to-sediment loading. This is because the origin of the thermal gradient is taken to be the sediment-air (water) interface. Thus, the calculated equilibrium and disequilibrium temperatures are a function only of the sedimentation rate and the thermal properties of the sediment column, which can be estimated from the stratigraphic record.

The flow of water through porous sediments affects their thermal history but is not taken into account in our model. Fluids move laterally and upward in a sedimentary basin as a result of compaction. This effect is not significant relative to conductive heating for the Illinois basin¹¹, which has sedimentation rates comparable with those in the Alberta Basin. On the other hand, gravity-driven groundwater flow may have a significant effect on sediment temperatures¹². The topography and water table of subaerial foreland basins slope gradually away from the deformation front toward the craton. The resulting flow pattern depresses the thermal gradients near the mountains and increases them at the cratonic limit of the basin¹³. Steady-state finite element models have been developed to estimate the effects of gravity-driven groundwater flow in the Alberta Basin^{14,15}. The thermal perturbations resulting from gravity-driven fluid flow are significant relative to the conductive effects of rapid subsidence and must be taken into account when modeling subaerial phases of subsidence. In the Alberta Basin subsidence is characterized by submarine and mixed submarine to non-marine sediments. Thus, a conductive heating model should yield a reasonably accurate thermal history during active subsidence, but would not be expected to yield an accurate picture of the present-day heat flow regime because of today's groundwater flow. A comprehensive model of the thermal history and present temperatures in the Alberta basin must include the effects of non-steady-state fluid flow as well as rapid sedimentation.

We have applied the thermal model to data from the Getty Union Oyster well in the Alberta Basin. The basin was produced by flexural bending in response to compressional tectonism between the Jurassic and Eocene. Before initiation of the foreland basin, the Hudsonian basement beneath the Alberta Basin was probably not affected by thermal events younger than ~1,700 Myr. Thus, the lithosphere can be assumed to have been in thermal equilibrium at the initiation of thrusting. The equilibrium gradient within the lithosphere is assumed to be 24 °C km⁻¹ at the surface of the crust. Radioactivity within the lithosphere is assumed to be distributed exponentially with a surface value of 2.5 μW m⁻³ and a decay constant (characteristic depth) of 14 km, resulting in a temperature at the top of the asthenosphere of 1,375 °C for a 125-km thick lithosphere. These values are consistent with average continental lithospheric

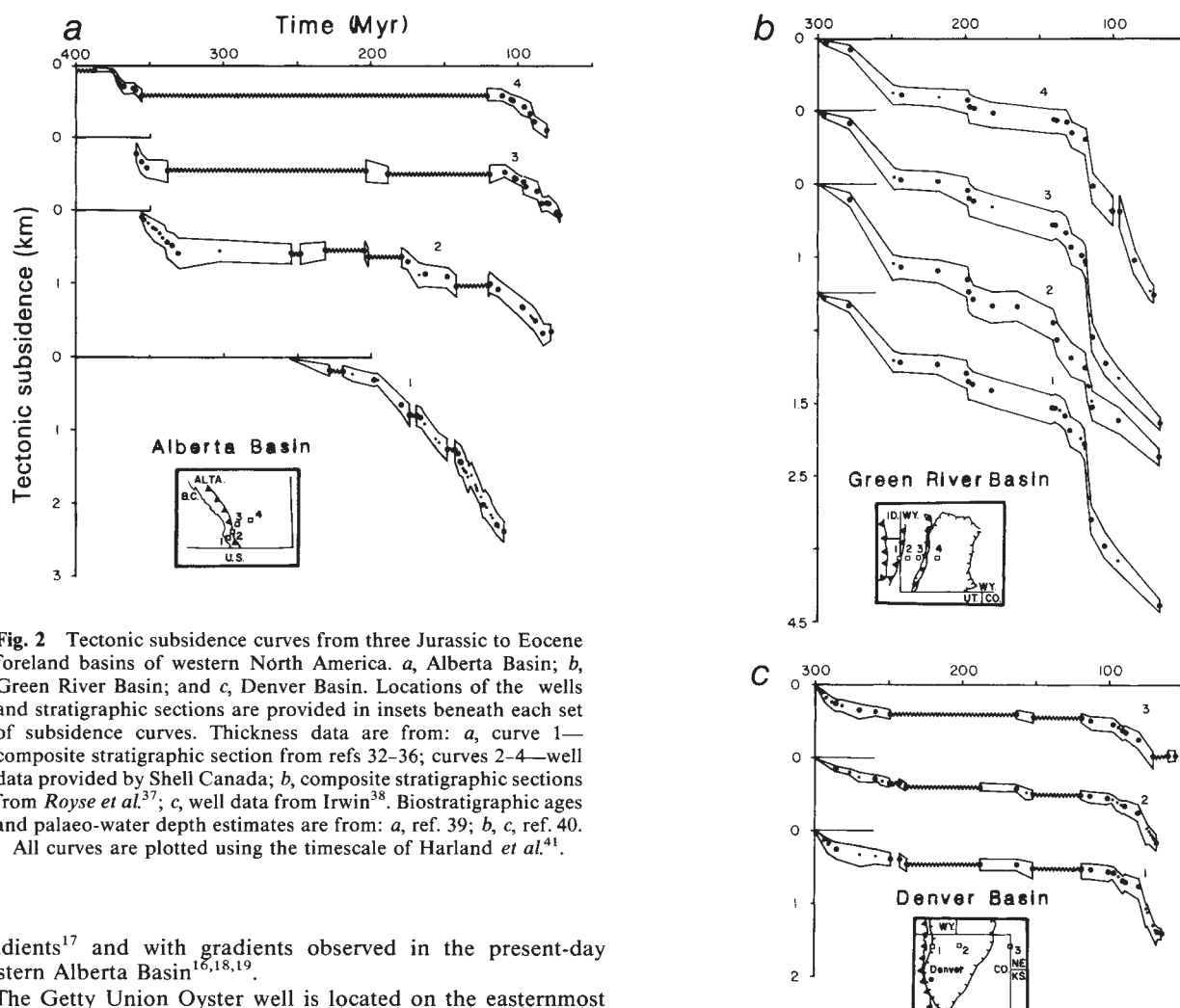


Fig. 2 Tectonic subsidence curves from three Jurassic to Eocene foreland basins of western North America. *a*, Alberta Basin; *b*, Green River Basin; and *c*, Denver Basin. Locations of the wells and stratigraphic sections are provided in insets beneath each set of subsidence curves. Thickness data are from: *a*, curve 1—composite stratigraphic section from refs 32–36; curves 2–4—well data provided by Shell Canada; *b*, composite stratigraphic sections from Royse *et al.*³⁷; *c*, well data from Irwin³⁸. Biostratigraphic ages and palaeo-water depth estimates are from: *a*, ref. 39; *b*, *c*, ref. 40. All curves are plotted using the timescale of Harland *et al.*⁴¹.

gradients¹⁷ and with gradients observed in the present-day western Alberta Basin^{16,18,19}.

The Getty Union Oyster well is located on the easternmost edge of the thrust and fold belt (Fig. 2, curve 2). Subsidence of the foreland began in the late Jurassic^{20,21} and ended at the end of the Cretaceous when thrusting overran this part of the basin. Because our model required that each sediment package be at least 100 m thick it was necessary to begin the modelling at the base of the middle Jurassic sediments (Fernie group); ~3.75 km of sediments accumulated between the middle Jurassic and the end of the Cretaceous (Fig. 3*a*). Our model predicts that the subsidence caused a negative thermal anomaly to grow through time to a maximum of ~−60 °C in the lithosphere and −11 °C within the sediments (Fig. 3*b, c*). Our model further predicts that after thrusting and subsidence ceased, the thermal anomaly would have decayed to −20 °C in the lithosphere.

The thermal anomaly within the sediments has an important effect on the calculation of maturation history within the deepest sediments modelled. The time-temperature index (TTI) of Waples²², calculated from the results of our thermal model, is lower than the TTI calculated assuming that thermal equilibrium is maintained throughout the subsidence history (Fig. 4*a*). The lower TTI values imply that the timing of peak oil generation would be delayed by ~4 Myr relative to that for equilibrium thermal gradients. The surface gradients are higher than predicted by the results of Hacquebard²³ and Majorowicz *et al.*¹⁹, who suggest that the gradient for the westernmost Alberta basin in Eocene time was about 22 °C km^{−1}. The calculation of the equilibrium and the time-dependent temperature histories from our model imply a higher degree of maturation than that which would be calculated using a constant temperature gradient of 22 °C km^{−1}. In general, the assumption of constant gradients through time in foreland basins^{19,24}, based on the present thermal gradients, is likely to yield maturation values that are too low

in the deeper parts of a foreland basin and too high in the shallower parts. Calculation of thermal gradients based on current maturation values in the western Alberta Basin by Beaumont *et al.*²⁵ yielded slightly higher gradients (26 °C km^{−1}) than those predicted for the Getty Union Oyster well by our model. Their thermal gradients yield correct final maturation values but may not accurately predict the timing of earlier maturation phases. Our modelling indicates that, in calculating maturation, it is not reasonable to assume that thermal gradients were constant throughout the history of foreland basin subsidence.

The decay of the thermal anomaly predicted by our model leads to thermal expansion and lower density within the crust and upper mantle. During subsidence of the basin the increasing buoyancy requires an increase in the driving force which produces subsidence. (The driving force is defined as the difference between the force required for the observed subsidence and the force exerted by the sediments.) The force is probably that portion of the downward force of the thrust loads which, because of the rigidity of the lithospheric plate, is transmitted to the location of the well being modelled. For the Getty Union Oyster well, the driving subsidence increases to ~122% of that which seems to be necessary in the absence of the thermal effects (Fig. 4*b*). In contrast to the calculation of thermal history, the driving force is dependent on several poorly constrained factors including palaeo-water depth, palaeo-sea level and the flexural response of the lithosphere to the sediment load. Of these effects, only palaeo-water depths have been included in the modelling. From middle Jurassic to late Cretaceous, the sea level was rising²⁶. Ignoring this change, therefore, results in an overesti-

mate of the subsidence resulting from the driving force. The reverse is the case when sea level fell from late Cretaceous to the present. Ignoring the flexural response of the lithosphere to sediment loading results in an underestimate of the driving force in the deeper part of the basin (the location of the Getty Union Oyster well). Airy isostasy overestimates the force of the sediments acting on the lithosphere directly beneath the greatest loads because the sediment load in the deepest parts of the basin is supported not only by the lithosphere directly beneath it but also by the adjacent lithosphere. Although the driving force is dependent on these corrections (sea level, flexural rigidity and water depth), the thermal history predicted by the model is not, and any correction made to the loading force curve calculated by the thermal model must also be made for the curve which ignores the temperature effect. Thus, the effect of the thermal history of the foreland basin on the buoyancy force, which is illustrated in the difference between the two curves in Fig. 4b, is independent of these uncertainties.

During depositional hiatuses and after cessation of loading, the heating of the lithosphere as the thermal anomalies decay will cause thermal expansion and uplift of the basin. In the Getty Union Oyster well, an uplift of ~ 5 m is predicted during the 6-Myr hiatus in the early Cretaceous. About 115 m of uplift is predicted since the end of subsidence (Fig. 4c). This accounts for only 12% of the present-day regional tilt from the foothills to the cratonic edge of the Alberta Basin. The remainder of the uplift may be derived from erosion of the mountain load. Alternatively, it could result from a decoupling of the load from the down-bent plate resulting from regional lithospheric heating in western North America²⁷. At the Getty Union Oyster well site, complete decoupling would require the removal of the total driving force (per unit area), 30.5×10^7 dynes cm^{-2} , resulting in 945 m of uplift. With the addition of thermal expansion, the total uplift is 1,060 m. This amount of uplift is quite close to the observed difference in elevation between the foothills and the cratonic edge of the basin^{24,28,29}. A decoupling of the load with the basin is consistent with the gravity modelling of McNutt³⁰. Her residual gravity anomaly map shows no paired anomaly in the Alberta foreland region, indicating that only local isostasy operates.

The heating of the lithosphere as the negative thermal anomaly decays also affects the temperature-dependent mechanical properties of the lithosphere. As the lithosphere gets hotter, its rigidity is reduced. The original thickness of the mechanical plate is assumed to have been 41 km. This is the apparent elastic thickness of old oceanic lithosphere³¹ and is also the elastic plate thickness used to model the Green River Basin². Our model predicts that heating of the lithosphere over the course of foreland subsidence reduces the elastic plate thickness by ~ 8 km (Fig. 4d). Because elastic rigidity is proportional to the cube of the elastic thickness, this result implies a 47% loss of flexural rigidity. Thus, the response of the lithosphere to loading is significantly affected, causing the plate to appear less rigid as subsidence continues. The magnitude of the loss in rigidity caused by heating is less than that predicted by the viscoelastic model of Beaumont¹ for the Alberta Basin but is similar in magnitude to that predicted by the thermally controlled creep model of Quinlan and Beaumont⁴. In our model the magnitude of the apparent viscosity of the lithosphere diminishes with distance from the load, resulting in a plate of variable rigidity. The effects of heating in foreland basin settings are complex and would be expected to result in a significant time-dependence in the response of the lithosphere to loading which should be taken into account in mechanical modelling of foreland basins. Neither the thermal heating nor any viscoelastic models predict the total loss of rigidity indicated by the current regional topography of the Alberta Basin. This implies that some event, not related to foreland basin formation, has subsequently affected the Alberta Basin.

In conclusion, our modelling of the thermal effects of episodic

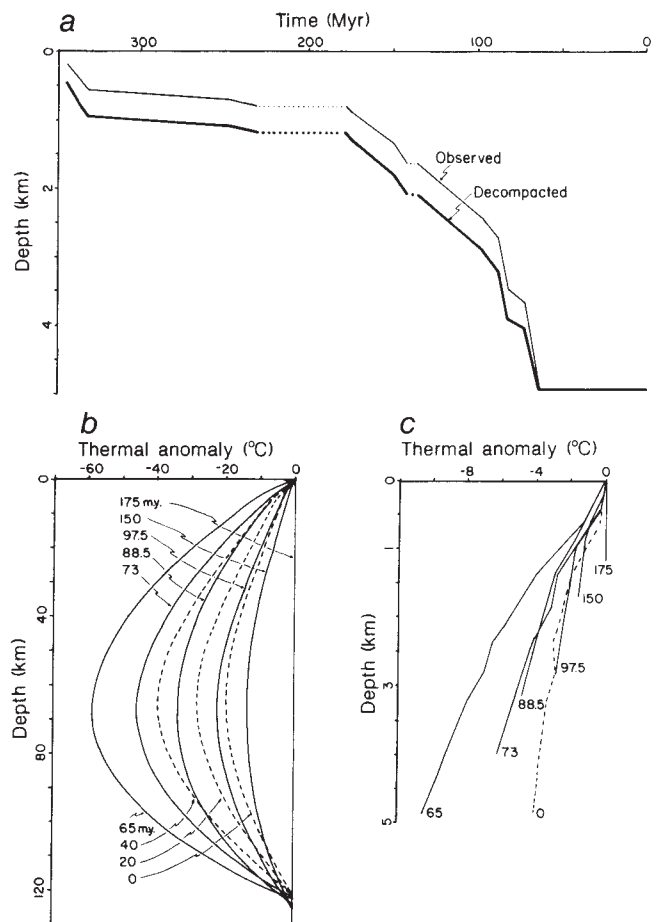


Fig. 3 *a*, Observed and decompacted subsidence history of the Getty Union Oyster well. The observed curve is a plot of cumulative thicknesses of the present-day sediments. The decompacted subsidence curve shows the subsidence of the basin with compaction of sediments taken into account. The decompacted subsidence curve specifies the subsidence history of the basin at the well, and serves as input for the thermal model. Profiles of thermal disequilibrium in the lithosphere (*b*) and the sediments (*c*) are plotted for specific times during and after active foreland basin subsidence. Foreland basin subsidence begins at 175 Myr, at which time there is no thermal anomaly. The thermal anomaly grows with time during foreland basin subsidence and decays after cessation of thrusting (65–0 Myr).

subsidence in a foreland basin shows that significant thermal anomalies are formed. The episodic subsidence history of the westernmost Alberta Basin, in which 3.75 km of sediments were deposited in 110 Myr, led to a thermal anomaly of -60°C in the lithosphere and an anomaly of -11°C in the sediments. While the anomaly in the sediments is small, and may be overwhelmed by local variations in temperature, it does represent a systematic effect which has been shown to be sufficient to reduce the final maturation level of the sediments. An implication of our model is that the heating of the lithosphere caused by the decay of the thermal anomaly is sufficient to modify significantly the results of the purely mechanical models that have been proposed for foreland basins. The growth and removal of the thermal anomaly within the lithosphere results in an increase in the force necessary to cause subsidence by $\sim 22\%$. The buoyancy force is manifested as thermally driven uplift during periods in which loading is not active, possibly causing basin-wide unconformities. Uplift of 115 m, after cessation of subsidence, is predicted for the Alberta Basin. A decrease in the rigidity of the plate by $\sim 47\%$ would appear as an apparent viscous relaxation of the plate. In many foreland basins, the

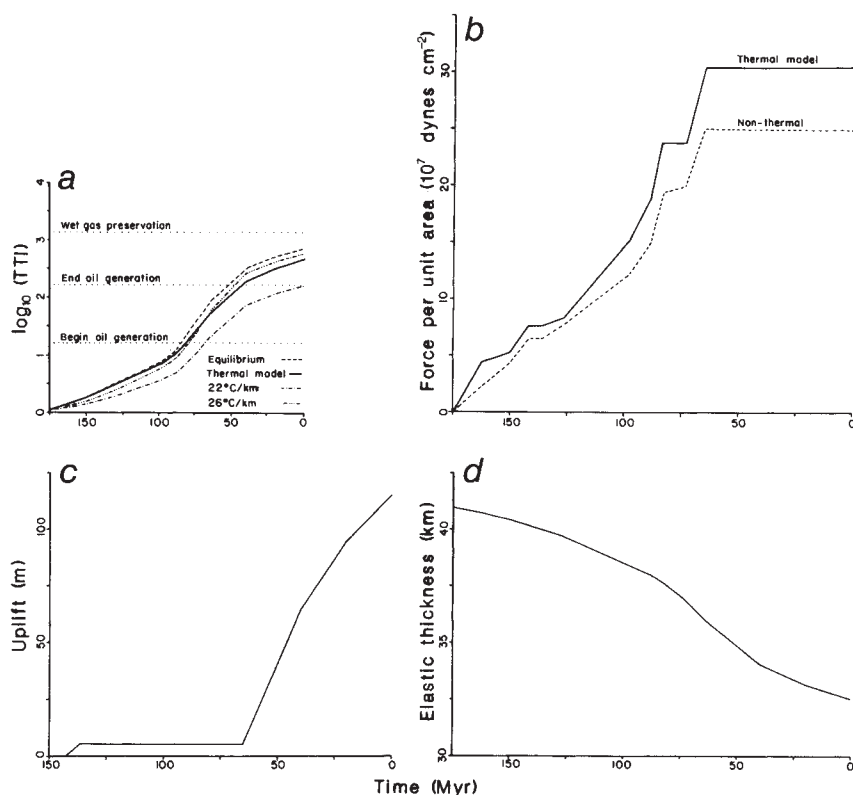


Fig. 4 *a*, Hydrocarbon maturation history predicted for Carboniferous strata in the Getty Union Oyster well. $\log_{10}$ TTI, as calibrated by Waples²², is plotted as a function of time. Solid (dotted) line, model result, taking (not taking) into account the thermal effects of rapid subsidence. *b*, The driving force per unit area applied to the lithospheric slab beneath the Getty Union Oyster well is shown for the case in which the thermal effects of subsidence are ignored and for the case in which the buoyancy force resulting from heating of the thermal anomaly is included. *c*, During times in which loading is not active, the buoyancy force causes uplift of the basin. The uplift during a 6-Myr depositional hiatus and after cessation of subsidence are plotted for the Getty Union Oyster well. *d*, Depth to the base of the elastic plate, assuming an initial elastic plate thickness of 41 km. The elastic thickness of the plate decreases over time as the negative thermal anomaly decays.

rates and/or magnitudes of subsidence are greater than those preserved in the Getty Union Oyster well. In such basins, the thermal anomalies and associated effects would be larger than those predicted for southwestern Alberta.

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³⁶Cl in a halite layer from the bottom of the Dead Sea

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Stable and radiogenic isotope compositions are commonly used to identify the solution from which minerals such as sulphate, carbonate and silicate were crystallized. Both the sources and the ages of evaporite deposits can be determined by measuring radioactive ³⁶Cl concentrations using new developments in accelerator mass spectrometry. Halite crystals found in saline sediments, which could not be measured by isotope techniques until this method was developed, are usually assumed to have precipitated from the lake water during former dry periods. In the case of the Dead Sea we tested this assumption by comparing the abundance of ³⁶Cl isotopes in a shallowly buried halite layer with that in the overlying water. We found that the ³⁶Cl/Cl ratio is significantly lower in the halite, which indicates that the salt did not crystallize from this water but from a solution originating from the Tertiary diapir underneath the sea or that the salt represents the top of the Mt Sdom formation diapir.

The two stable chlorine isotopes (^{35}Cl and ^{37}Cl) show a small isotope effect of the order of 3‰ (ref. 1) which can be used in studies of both recent and ancient evaporite deposits. A third isotope, ^{36}Cl , which is cosmogenic with a half-life of 301,000 yr, can be used not only as a tracer of the depositional environment but also as the basis of a dating method for halite in the Pleistocene².

The determination of ^{36}Cl in natural water has already been used to demonstrate the influence of 'ancient' salts on present water systems³. Old salt sources (older than several half-lives) are characterized by very low $^{36}\text{Cl}/\text{Cl}$ ratios⁴ (these may be $<0.5 \times 10^{-15}$). The ratio probably never falls below this value because of a second mode (non-cosmogenic) of ^{36}Cl generation involving the capture of neutrons originating from the interaction of α particles on light elements. In some cases the ratio can be higher because of local enrichment of uranium and thorium⁴.

The Dead Sea is located in the Jordan rift zone, which is the northern extension of the Syro-African Rift system. We found a layer of massive halite⁵⁻⁷ under a thin layer of soft sediments in the northern Dead Sea. Although the thickness of the halite layer was not determined, its continuity was demonstrated by extensive coring, the almost homogeneous distribution of trace elements⁸ and by seismic profiles (Z. Ben Avraham, Y.L. and J. K. Hall, in preparation).

We analysed a halite sample recovered from the bottom of a 0.4-m-long core taken near Ein Gedi at a depth of 250 m. Associated with the sample was a carbonate mud (70% aragonite, 30% calcite) which yielded a ^{14}C age of 500 ± 500 yr BP (taking into account that present-day Dead Sea water has an apparent age of 2,000 yr; ref. 6).

The halite was dissolved in water and its Cl^- precipitated with Ag^+ and purified by dissolution in NH_3 before the final reprecipitation as AgCl . We measured ^{36}Cl in the AgCl target using the accelerator mass spectrometer system at the Rehovot 14UD Pelletron Tandem Laboratory⁹. Two samples run on different days yielded $^{36}\text{Cl}/\text{Cl}$ values of 6 ± 3 and $7 \pm 5 \times 10^{-15}$. These values should be compared with those of Dead Sea water and recent (1981) halite which precipitated on a rope suspended in the Dead Sea water column (Table 1).

The distinct difference between the $^{36}\text{Cl}/\text{Cl}$ ratios in the samples obtained from Dead Sea water and in those of the salt sampled from the halite layer at the bottom of the Dead Sea exclude the possibility that it precipitated from Dead Sea brine either recently or during the past few tens of thousands of years. Yet the age of the chlorine in the water column was found to be between 19,000 and 25,000 yr, based on the ^{36}Cl concentration and an input model presented by Paul *et al.*³. On the other hand, the $^{36}\text{Cl}/\text{Cl}$ ratio is very similar both to that of Mt Sdom halite taken from a depth of 2,000 m and that of the Ashlag Springs, which represent solutions associated with the Mt Sdom formation (Table 1). Although the last three values in Table 1 are

higher than ratios measured in some old deposits⁴, they are the lowest determined in our laboratory. These values can be explained either by local enrichments of U-Th as mentioned above, or by experimental effects such as cross-contamination during sample preparation or ion source operation.

Several models can be proposed to explain the origin of the halite layer based on its $^{36}\text{Cl}/\text{Cl}$ ratio and on other geochemical and geophysical evidence: (1) The halite is the topmost layer of a salt diapir¹⁰. Neev and Hall¹⁰ believe that the diapiric structures detected in seismic profiles indicate the occurrence of rock salt masses of the Mt Sdom formation in different areas of the northern basin of the Dead Sea. (2) The salt was deposited recently from interstitial chloride-bearing solutions that diffused upward from the underlying Mt Sdom formation and not from the contemporary Dead Sea brine. This model can explain the co-occurrence of 'old' salt with sediments whose young age (500 ± 500 yr BP) is indicated by the ^{14}C dating of the carbonate fraction. If one accepts this model and the observations of Ben Avraham *et al.* (in preparation) that the salt layer covers most of the floor of the northern Dead Sea, one has to postulate that a brine is diffusing upwards under most of the present-day Dead Sea bottom sediments. According to this model, most of the chlorine dissolved in the present-day Dead Sea brine is derived from the dissolution of rock salt. This is consistent with the findings of Paul *et al.*³, based on Cl and ^{36}Cl balances, that the ratio of the Cl^- derived from rocks to the Cl^- derived from rain is 100 for the Dead Sea. (3) The halite layer was precipitated following an event during which concentrated saline solutions¹¹ of the Ashlag Spring type flowed to the bottom of the Dead Sea in large quantities and remained there because of their high density. Table 1 demonstrates that such solutions are characterized by low $^{36}\text{Cl}/\text{Cl}$ ratios.

These models cannot be differentiated by geochemical methods because in all three, the halite is either part of the Mt Sdom formation or derived from a solution of its salt. Another geochemical parameter that has been suggested as an indicator of the salt's origin is the Cl/Br ratio. This ratio, found to be 3,500–5,300 in the halite layer, is similar to that in Mt Sdom salt and significantly different from the value (1,100) found in halite precipitating from modern Dead Sea water⁸. On the basis of such evidence, Bloch and Picard¹² suggested a Neogene age (the age of Mt Sdom salt) for the halite layer. However, Cl/Br ratios alone are no more than indicative, as halite is known to lose some of its bromide during diagenetic changes.

We can conclude that: (1) the halite does not represent a Dead Sea water precipitate; (2) the halite is associated with Mt Sdom salt, either by being part of the Mt Sdom formation or by originating from a solution whose Cl^- is derived from Mt Sdom salt; and (3) the association of the old salt and young CaCO_3 in the halite layer may favour the second or third hypothesis. Further measurements of ^{36}Cl in interstitial water and in additional halite samples may elucidate the processes that led to the formation of the halite layer studied.

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Table 1 Results of ^{36}Cl determination of samples from the Dead Sea area

	$^{36}\text{Cl}/\text{Cl}^*$ (10^{-15})
Dead Sea	
Surface	17 ± 4
80 m†	17 ± 2
310 m	20 ± 4
Ashlag Spring	4 ± 2
Mt Sdom salt	5 ± 2
Halite layer (Dead Sea core)	6 ± 3

* The value shown is the weighted mean of independent measurements $\pm$ variance of the mean.

† From halite crystals deposited on a rope suspended in Dead Sea water.

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Carbon-isotope events across the Precambrian/Cambrian boundary on the Siberian Platform

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Variations of marine isotopes with time have been observed through the Phanerozoic^{1,2}, in association with some period boundaries: Pleistocene/Holocene³, Cretaceous/Tertiary⁴, Permo-Triassic^{5,6} and Frasnian/Famennian⁷. Most of these changes are associated with extinction events, reflecting changes in life on Earth. One of the major biological changes in Earth's history occurred near the end of Proterozoic time, with widespread increase of biomineralization and the appearance of shelly fauna⁸⁻¹⁰. We present here an initial survey of carbon isotope ratios in a section on the Siberian Platform that spans the Proterozoic/Palaeozoic boundary. After a high of $\delta^{13}\text{C} = +3.4\%$, 15 m below the boundary, $\delta^{13}\text{C}$ drops sharply in two cycles across the boundary, to $\delta^{13}\text{C} = -2\%$ near the end of the Tommotian Stage. These variations suggest an initial bloom of biomass in late Vendian time corresponding to the dramatic diversification that must have preceded the widespread appearance of new taxa in the Cambrian fossil record.

The section studied comprises 250 m of carbonate rocks that include the Yudoma Formation of late Vendian age and the Pestrotsvet Formation of Tommotian age along the Aldan River (Dvortsy section, 57.47° N, 129.30° E, 650 km south of Yakutsk) on the Siberian Platform in Yakutia¹¹⁻¹³. The Vendian/Tommotian boundary, close to the top of the Yudoma Formation, is a candidate for designation as a stratotype of the Precambrian/Cambrian boundary¹⁴. Arguments have been advanced against this selection¹⁵⁻¹⁷, but our concern here is with the fossil record rather than the formalities of stratigraphy. Consequently in the following discussions we will informally refer to the Vendian/Tommotian boundary as the Precambrian/Cambrian (P/C) boundary. The Dvortsy sequence is transgressive over a shelf of the Archaean Aldan Shield, and this is part of a worldwide transgression that continues through Tommotian time¹⁸. The rocks range from very fine (<10 μm) pelletal dolomite to medium-coarse (100-150 μm) xenomorphic dolomites. Part of the topmost Vendian sequence is dolomite replacing earlier oolitic sediments. As one approaches the suggested P/C boundary from below, de-dolomitization and staining with iron oxide indicate an unconformity, suggested by Khomentovskiy *et al.*¹¹. Our Cambrian samples were also dominantly dolomite, although this section is often described as limestone¹¹.

Our samples were obtained as part of IGCP Project 29, and were collected in a similar fashion to a previous set of Tommotian samples from along the Lena River, studied for magnetostratigraphy by Kirschvink and Rozanov¹⁹. Palaeomagnetic studies of the new samples are in progress by Kirschvink.

CO_2 for mass spectrometry was generated by the method of Magaritz and Kafri²⁰. CO_2 generated by calcite in the de-dolomite was removed, and then the remaining dolomite reacted to provide the measured sample. Although the section has suffered dolomitization, probably in an early diagenetic stage, we know from much younger dolostones that the dolomite preserves the original $\delta^{13}\text{C}$ values^{21,22}.

From the base of the Dvortsy section $\delta^{13}\text{C}$ becomes gradually more positive, from $\delta^{13}\text{C} = -4$ to $+3.4\%$ (Fig. 1). The most

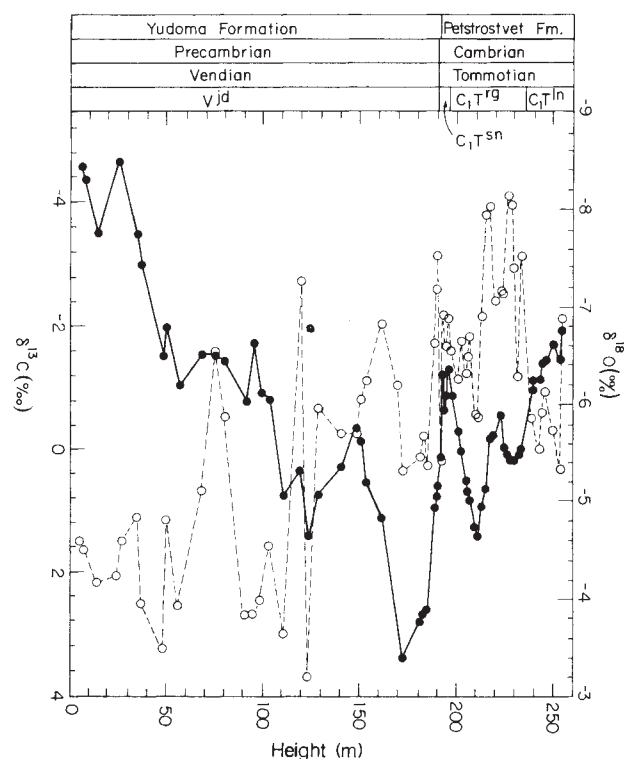


Fig. 1 Profiles of $\delta^{13}\text{C}$ (●, solid line) and $\delta^{18}\text{O}$ (○, dashed line) at the Dvortsy section, Aldan River, Yakutia. The metre scale corresponds to the standard scale scribed on the outcrop, which starts at an arbitrary zero about 10 m above the unconformity on the Archaean; the isotope profile begins at +10 m on that scale, or about 20 m above the unconformity. Stratigraphical zone symbols¹¹: Vjd, Vendian Yudoma Formation; C₁T^{sn}, Cambrian Tommotian Pestrotsvet Formation, *A. sunnaginicus* zone; C₁T^{sg}, *D. regularis* zone; C₁Tⁱⁿ, *D. lenaicus* zone.

positive samples are found at about 170 m, 15 m below the P/C boundary. From this point, a decline of $\delta^{13}\text{C}$ to a minimum of $\delta^{13}\text{C} = -1.3\%$ crosses the P/C boundary, as well as the rest of the Yudoma Formation and the *Aldanocyathus sunnaginicus* zone (Fig. 1: C₁T^{sn}, of lower Tommotian age) of the Pestrotsvet Formation. From this point, $\delta^{13}\text{C}$ rises sharply by 2‰ within a 15-m section of the *Dokidocyathus regularis* zone (Fig. 1: C₁T^{sg}, of middle Tommotian age), and then decreases by 3.3‰ to the end of the analysed section about 10 m below the Tommotian/Atdabanian boundary.

The rise of 7.5‰ in $\delta^{13}\text{C}$ (Fig. 1) within the Vendian, is at an average rate of change of $0.04\% \text{ m}^{-1}$. The second rise, in the *D. regularis* zone (C₁T^{sg}) is of the order of $0.2\% \text{ m}^{-1}$, about five times as fast as in the late Vendian, on the admittedly tenuous assumption of a uniform sedimentation rate. The drop of $\delta^{13}\text{C}$ across the P/C boundary is at a faster rate, of $0.5\% \text{ m}^{-1}$. Part of this section may be missing at the unconformity, which may mean that the drop is at a similar rate to the previous Vendian increase. Because each of these changes continues over a thick sequence, it is likely that none of them is caused by an instantaneous mechanism. The carbon isotope variations reflect corresponding variations of $\delta^{13}\text{C}$ in HCO_3^- in shelf waters, which presumably equals that of the surface ocean water mass. Several models have been proposed to explain variations of $\delta^{13}\text{C}$ in surface ocean waters. For this case, we can ignore those models dependent on the mass of land flora. The remaining models are: (1) changes in biological productivity in the surface ocean²³; (2) changes in vertical circulation rate for the whole ocean²⁴; (3) changes in the fractional preservation of organic carbon on the sea floor, caused by expansion and contraction of the oxygen-minimum zone²⁵.

Although we have only a single profile, previous experience with profiles of $\delta^{13}\text{C}$ at other boundaries suggests that at least the general trends of variation represent worldwide oceanic events, rather than simply basinal changes.

The number and size of phosphorite deposits near the P/C boundary is unequalled at any other time in the geological record^{12,26,27}. Phosphorite deposition suggests increased biological productivity, which (model (1)) should increase $\delta^{13}\text{C}$ in these surface waters. Cook and Shergold²⁶ connect this burst of phosphorite deposition with the explosive faunal diversification in early Cambrian times, including taxa with phosphatic skeletons. Their time control for the phosphate event(s) is necessarily imprecise, and carbon isotope profiles may eventually refine this course of events. In accordance with the above model, the two periods of increased $\delta^{13}\text{C}$ correspond to increases in biological activity. The first rise of $\delta^{13}\text{C}$ occurs in the Vendian system, which has little skeletal fauna and may correspond to a time of diversification, the descendants of which appeared at once in the early Cambrian. This general development was probably interrupted by a decline of productivity, revealed by the reversal across the P/C boundary, which recovered only in the *D. regularis* zone (middle Tommotian). A second decline continued to the top of our record of $\delta^{13}\text{C}$ (Fig. 1).

The changes of $\delta^{13}\text{C}$ in Fig. 1 probably represent a final episode of a long record of generally high $\delta^{13}\text{C}$ in late Proterozoic time, as displayed in thick sections in Svalbard and Greenland (A. H. Knoll, personal communication). In that view, the low $\delta^{13}\text{C}$ with which our shorter section begins would have been only a temporary drop to a low value.

On a section that is still shorter than ours, $\delta^{13}\text{C}$ across a zone designated as the P/C boundary at two localities in China shows a sharp negative excursion within 20 cm of the boundary, associated with a possible iridium anomaly^{28,29}. However, the levels at which the P/C boundary has been placed in China and Siberia are probably not stratigraphically correlative. In any case, our sampling would probably have missed so sharp an excursion as found in China.

The $\delta^{18}\text{O}$ values in Fig. 1 have a negative trend, from a mean of -4% in the first 150 m of our Vendian section, to about $\delta^{18}\text{O} = -7\%$ in the rest of our Vendian and Cambrian section. No major change occurred at the P/C boundary. The correlation of $\delta^{18}\text{O}$ with $\delta^{13}\text{C}$ is -0.36 , whereas a positive correlation would have been expected if both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ had been distorted by freshwater diagenesis. As with other dolomites, one cannot be sure that they preserve an original record of $\delta^{18}\text{O}$. Further analyses, especially of phosphate $\delta^{18}\text{O}$ (ref. 29), may help to explain the variations in $\delta^{18}\text{O}$.

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Note added in proof: The recent paper by Tucker³¹ gives a carbon isotope profile across the P/C boundary in the Anti-Atlas of Morocco. In that section the boundary is undefined within a 200-m section, which in turn forms part of a 300-m interval for which Tucker has no $\delta^{13}\text{C}$ data. But Tucker's $\delta^{13}\text{C}$ values rise from -3% below this interval to $+2.5\%$ above this interval, which probably corresponds to the late Vendian rise in Fig. 1 above. A more complete profile of $\delta^{13}\text{C}$ in the Moroccan section may give a precise correlation with the Siberian section.

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Radiolarian and silicoflagellate response to oceanographic changes associated with the 1983 El Niño

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Strong seasonal signals in fluxes and composition of siliceous microfossils have been recorded in sediment traps from two sites in the equatorial Pacific, deployed from December 1982 to March 1984. During the early part of the sampling period, the 1982-83 El Niño event had a profound effect on the radiolaria and silicoflagellates within these two areas. During the El Niño, the radiolarian trap assemblages at Site C (1° N, 139° W) most resembled faunal assemblages in western equatorial Pacific sediments, whereas the trap assemblages resembled equatorial divergence sediments in the latter half of the period. At Site S (11° N, 140° W), the radiolaria and silicoflagellate species can be correlated with organic carbon fluxes. In general, silicoflagellate shell fluxes are correlated to total opal fluxes where radiolarian fluxes do not exhibit this relationship. Approximately 20-25% of the total count of radiolaria in traps are not present in underlying sediments with a significant loss of the weakly silicified forms. However, the comparison of trap sample assemblages with underlying sediments reported here shows that dissolution does not alter relative abundances of the fossil species used in palaeoclimatic reconstructions. A significant difference is observed between the silicoflagellate trap assemblage and the underlying sediment assemblage.

The samples used in this study were collected with the Oregon State University single-cone traps equipped with five-cup sample changers¹. The sampling period was 7 days for the first cup and ~100 days for each of the other four cups. At Site C the traps were deployed at 1,095, 1,895 and 3,495 m, at Site S, 700, 1,600 and 3,400 m. Microfossil assemblages were examined to determine the changes in the fluxes and species abundance associated with the marked changes in organic carbon fluxes measured in the sediment traps. In addition, we wanted to determine if the known distributions of radiolaria and silicoflagellates in surface sediments of the equatorial Pacific could be used to infer the

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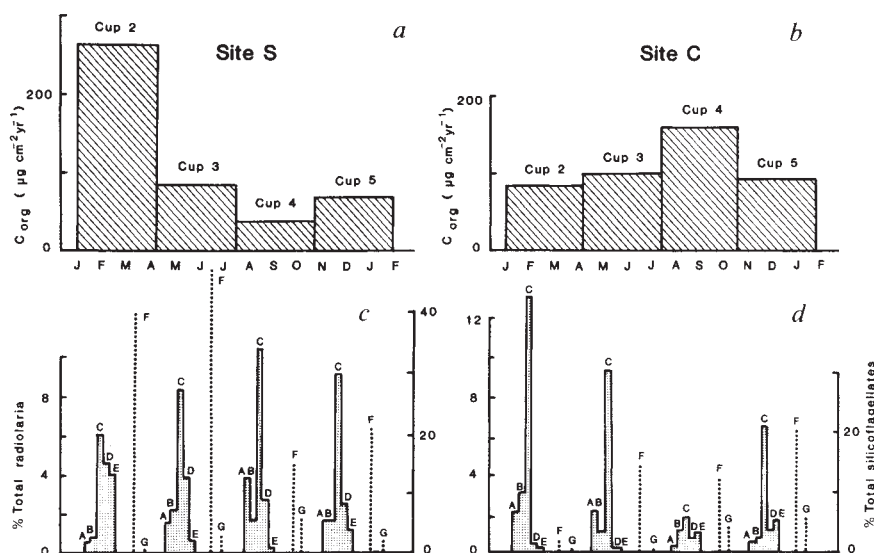


Fig. 1 Organic carbon fluxes at: *a*, Site S; and *b*, Site C for 1983; months abbreviated as single letters. Relative abundances of radiolarian (labelled A to E) and silicoflagellate (F and G) species at Site S (*c*) and Site C (*d*). Scale for radiolaria is to the left of frames *c* and *d*, silicoflagellates to the right. A, *H. enthacanthum*; B, *D. tetrathalamus*; C, *T. octacantha/O. stenozona*; D, *P. minytorax*; E, *B. auritus-australis*; F, *D. pulchra*, and G, *D. calida*.

nature of oceanographic conditions associated with changes in organic carbon transported to the deep sea.

The $>63\ \mu\text{m}$ fraction from sample splits was used to make quantitative radiolarian microfossil slides using a technique described elsewhere². The average total count of radiolaria in each of the 24 trap samples was 750 with 142 species identified. The $<63\ \mu\text{m}$ fraction from each of the samples was used for preparing silicoflagellate slides. Approximately 250 silicoflagellates were counted in each sample with three species identified.

The estimated fluxes of radiolarian shells in the $>63\ \mu\text{m}$ fraction range from 65 to 332 shells $\text{cm}^{-2}\text{yr}^{-1}$ at Site S and from 89 to 558 shells $\text{cm}^{-2}\text{yr}^{-1}$ at Site C. These fluxes are a factor of 3–10 less than those measured by Takahashi³ who studied radiolaria in both the coarse and fine fractions at stations in the tropical Pacific and Atlantic. Comparison of individual radiolarian species fluxes estimated in the Site S and C traps and those of Takahashi³ are in good agreement. For example, the maximum fluxes measured for the species *Heliodiscus asteriscus*, *Spongaster tetras tetras* and *Pterocorys zancleus* are 2, 6, and 18 shells $\text{cm}^{-2}\text{yr}^{-1}$ as compared with other trap experiment values for these species of 1–2, 1–2 and 7–26 shells $\text{cm}^{-1}\text{yr}^{-1}$ respectively³.

The organic carbon flux data used in this study is from ref. 4. The flux of organic carbon shows a marked response to the oceanographic events of 1982–83. During the early part of the trap deployment, organic carbon flux was at a minimum at Site C (Fig. 1). This would be expected from the general picture of reduced equatorial circulation associated with the El Niño event of 1983. However, the carbon flux measured at Site S at 11°N was extremely high ($\sim 270\ \mu\text{g cm}^{-2}\text{yr}^{-1}$). This flux is one of the highest 3-month values measured in the open ocean by the OSU trap programme (J. Dymond, personal communication). As the year progressed, organic carbon flux in the Site S traps fell dramatically to levels below $80\ \mu\text{g cm}^{-2}\text{yr}^{-1}$, whereas at Site C, the carbon flux increased to a maximum value (Fig. 1). On an annual basis, the carbon flux was higher at Site S than at Site C. This is contrary to the expectation that Site C, within the equatorial divergence, would have the higher organic carbon flux as compared with Site S at the edge of the equatorial productivity belt.

We have found that the relationship between the fluxes of either total radiolarian shells or individual species fluxes is not as closely related to the flux of organic carbon ($r^2 = 0.04\text{--}0.49$) as the relationship between radiolarian species composition and organic carbon fluxes ($r^2 = 0.17\text{--}0.87$). Thus, we present here only the results from the comparison of radiolarian species composition and trap-measured organic carbon flux.

The results from the analysis of radiolarian species composition in the sediment trap samples are also shown in Fig. 1. The percentages of five radiolarian species are displayed for each of the four seasonal samples. The five species of radiolaria were selected for detailed study based on their distribution in surface sediments from the equatorial Pacific^{5,6}, and all are important components of the deep-sea sediment thanatocoenoses.

Tetrapyle octacantha/Octopyle stenozona (counted together in both sediment traps and surface sediment samples) are one of the more abundant species of the equatorial region with highest abundances found in the eastern subtropical Pacific. *Hexacanthium enthacanthum* is found in the eastern equatorial Pacific in lower abundances than *T. octacantha/O. stenozona*, and also in the California Current region. *Didymocorys tetrathalamus* is also a tropical species but, unlike the previous two species, is found in highest abundances in sediments of the western Pacific.

Two other species were examined because of their association with eastern boundary currents and the equatorial divergence zone. *Pterocorys minytorax* is found in highest abundance in the eastern equatorial Pacific and Peru Current region and in minor percentages off the southern coast of Baja California and Central America. *Botryostrobus auritus-australis* is found in the Peru and California Currents and in a narrow band along the Equator⁶. Thus, we hypothesize that increased equatorial divergence and its associated productivity would favour the increased abundance of these two radiolaria as compared with the other

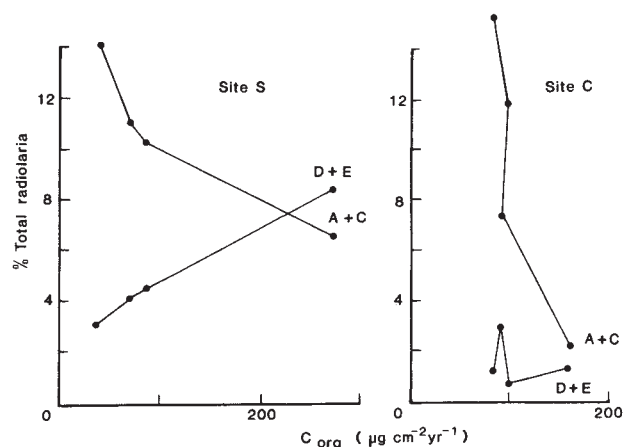


Fig. 2 Relative abundance of radiolarian species versus organic carbon flux in the near surface traps at Site C and Site S. Letters represent the same species as in Fig. 1.

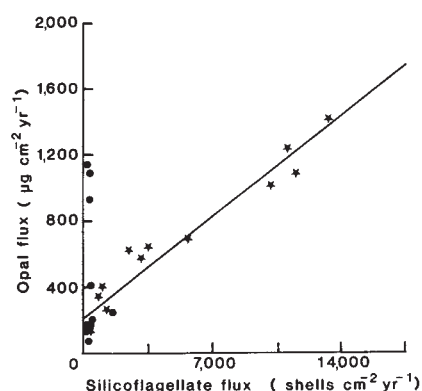


Fig. 3 Combined biogenic silica flux from all traps at all depths versus the total flux of silicoflagellates. ★, Site C; ●, Site S. Correlation coefficient of data (excluding the very high opal flux from cup 2 at Site S) equals $r^2 = 0.94$.

subtropical forms. The differing radiolarian fauna found in the eastern equatorial and western Pacific would also be useful in identifying different circulation patterns associated with the unusual oceanographic events of 1983 (refs. 7–9).

The data from the trap deployments support these hypotheses. A simple relationship is observed between the percentages of the inferred 'productivity' species *P. minyathorax* and *B. auritus-australis* and the flux of organic carbon with increased percentages of these species corresponding to increased organic carbon flux (Fig. 2). The species *T. octacantha*/*O. stenozona* decreases in abundance with increasing organic carbon flux. A forced negative correlation between these species is not likely for two reasons. First, these species make up <20% of the total radiolarian count in which 142 species were identified. Second, up to 20% of the total count was not identified. Thus, at Site S, the marked changes in organic carbon flux due to the El Niño even of 1983 are reflected by changes in species abundance (Fig. 2).

At Site C, the relationship between carbon flux, radiolarian fauna and oceanographic events differs from that at Site S. The changes in the 'productivity' species shows very little correlation with the organic carbon flux ($r^2 = 0.11$). We feel that this reflects the marked faunal abundance change which occurred during the sampling period. Twenty-five species, including *T. octacantha*/*O. stenozona* and *D. tetrathalamus*, have their highest abundance in cup 2 at Site C.

Sea-surface temperatures at Site C show a marked warm anomaly during the early part of the deployment, returning to near normal conditions by July of 1983 (ref. 4). This anomalously warm water, which was observed at the Equator throughout the Pacific^{7–9} (but not at Site S), was a result of anomalous eastward transport along the Equator. The relative abundance of radiolaria at Site C indicates the presence of western Pacific waters during the early part of 1983. Comparison of the radiolarian fauna with surface sediment distributions shows that the sediment samples most similar to the fauna found in cups 2 and 3 of Site C are from the far western equatorial Pacific (Table 1). The fauna in cups 4 and 5 of Site C and all the cups of Site S most resemble samples from the eastern equatorial Pacific and Panama Basin (Table 1). Thus, at Site C where a major abundance change in the fauna occurred during the deployment, less correlation is found between the radiolarian fauna and organic carbon flux changes.

Silicoflagellates also demonstrate a marked response to the El Niño event. Seasonal silicoflagellate fluxes range from 73 to 1,277 shells $\text{cm}^{-2} \text{yr}^{-1}$ at Site S and 365 to 14,600 shells $\text{cm}^{-2} \text{yr}^{-1}$ at Site C (Fig. 3). A similar range in fluxes has been reported in traps from the eastern equatorial Pacific¹⁰.

In general, silicoflagellates are a minor constituent (~1%) of the opal flux. This is especially true at Site S where the <63 μm

Table 1 Comparison between sediment trap and surface sediment radiolarian assemblages

Trap sample	Surface sediment*	Similarity measure†
Site S (700 m) (11° N 140° W)		
Cup 2	Y71-9-85 (5° S 087° W)	0.89
Cup 3	V24-046 (1° N 087° W)	0.95
Cup 4	Y71-9-91 (6° S 115° W)	0.93
Cup 5	V18-324 (8° N 107° W)	0.89
Site C (1,095 m) (1° N 139° W)		
Cup 2	RC13-23 (0° N 175° W)	0.97
Cup 3	RC10-136 (10° S 154° E)	0.96
Cup 4	V21-207 (0° N 100° W)	0.69
Cup 5	RC10-249 (7° N 087° W)	0.91

* Samples listed are surface sediment samples which had the highest similarity value with the listed trap sample. Samples were selected out of a set of 191 samples. Thirty-four species were used and the average sediment count was 550 radiolarians per sample.

† The cos θ measure of similarity was used. This index ranges in value from 0.0 to 1.0 with a value of 1.0 indicating that all inter-species ratios in the two samples being compared are identical.

fraction and opal flux are dominated by diatoms. However, at Site C there is a marked increase in the silicoflagellate to diatom ratio in the fine opal fraction as compared with Site S. Unlike the flux of radiolaria shells in the coarse fraction, the flux of silicoflagellates are highly correlated ($r^2 = 0.95$) with the flux of biogenic silica (Fig. 3). The very high opal flux measured in cup 2 of Site S is the result of a large increase in the diatom species *Thalassionema bacillaris* and *Thalassiothrix longissima*, and is not reflected by an increase in silicoflagellates.

The silicoflagellate assemblage at both sites is dominated by *Dictyocha messanensis* a species common throughout the North Pacific¹¹, *Distephanus pulchra*¹² and *Dictyocha calida*. *Distephanus pulchra*, a species associated with high productivity in the Gulf of California^{13,14}, is ~15% of the assemblage at Site C and 30–50% at Site S, mirroring the shift of 'productivity' species in the radiolarian results.

The succession of the silicoflagellates at both sites supports the results of the radiolarian studies. At Site S, the three dominant equatorial silicoflagellate species are present throughout the deployment period. Variations in *D. calida*, typical of the warmer waters of the equatorial Pacific¹¹, follows the temperature patterns observed at this site. During the early part of the year when temperatures were at a minimum, *D. calida* is found in lowest abundance (Fig. 1). As temperatures increased during the spring and summer this species increased in relative abundance. The species *D. pulchra*, associated with high productivity, is found in highest abundance in cup 3, which has the highest organic carbon flux (Fig. 1). The percentage of this species decreases 50% in the latter part of the year.

At Site C, there is a marked change in the silicoflagellate assemblage during the sampling period. *Dictyocha calida* is not found during the first half of the sampling period and reaches abundances similar to that at Site S in cup 5 (Fig. 1). *Distephanus pulchra* is also found in very low abundances in cup 2 (<2%) as compared with the latter sampling intervals. Thus, the oceanographic events of 1983 are reflected in significant faunal and floral change within the equatorial band but not at 11° N.

Results from the analysis of the Sites S and C traps differ from previous studies in two important ways. It has been suggested that dissolution does not significantly alter the sinking radiolarian population³ but comparison of trap and surface sediment populations showed that there were significant differences between the two¹⁵. We found that up to 25% of the settling radiolaria are rare in the underlying sediments and significant loss of these weakly silicified species was noted within the vertically distributed traps. Three species (*Dictyophimus* spp., *Lithomelissa thoracites* and *Peridium* spp.) account for most of the weakly silicified fraction with a combined average of 14% of the total count at Site S and 17% at Site C. These three species are important components of the radiolarian assemblage found in sediments in the Gulf of California¹⁶ but are not common in sediments from the equatorial Pacific. More importantly to palaeoclimatic and palaeoceanographic studies, dissolution does not seem to alter selected microfossil components within the radiolarian thanatocoenoses. Comparison of our trap sample species abundances and an adjacent surface sediment sample from core RC11-210 (1° N 140° W) using only species studied in palaeoclimate reconstructions^{5,17} shows very high similarity. The comparison was made by taking a weighted sum (weights were set equal to the radiolarian flux in each sample) of radiolarian species abundance in the four samples from the shallowest trap at Site C. The calculated similarity with a near surface sample of core RC11-210 was 0.91. Samples from the Site S traps could not be compared with sediments from the deployment area as this region of the Pacific is characterized by extensive sediment reworking so that Eocene to late Miocene radiolarian species are found in the near surface sediments.

In contrast, silica dissolution has a significant impact on the silicoflagellate assemblage. *Distephanus pulchra* is an important component in both the Site S and Site C traps. However, this species is not found in the surface sediment at either of the two sites studied and Poelchau¹¹ and D.M. (unpublished data from Manop Sites H and M) only found it in high abundance in northeastern equatorial Pacific sediments beneath areas of high opal sedimentation rates. This indicates that the distribution of *D. pulchra* in deep-sea sediments is controlled largely by dissolution, and ecological interpretation of this species from deep-sea sediments must be made with caution.

This study supports the contention that sediment trap studies provide important information about the transfer of biogenic components from their production in the near surface ocean to final burial in deep-sea sediments. Also, the study of microfossils in traps not only provides valuable information about the ecology and surface ocean conditions reflected in the fossil species preserved in deep-sea sediments, but in turn microfossil studies provide important information needed to interpret processes controlling the transfer of organic components to the deep-sea.

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A new prokaryote containing chlorophylls *a* and *b*

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Prochlorophyta, suggested as a new division of prokaryotes¹, lack phycobillin pigments characteristic of cyanobacteria, but contain chlorophyll *b* as well as chlorophyll *a*, characteristic of green algae and higher plants. Since the description of *Prochloron didemni* as the type species for this division², no other genera or species have been added to the group. The only published accounts of *Prochloron* are obligate symbionts of didemnid ascidians³, which are difficult to grow in the absence of their hosts⁴. Consequently, research on their cell composition and physiology has been handicapped. Here, we report a second prochlorophyte. This organism is one of the dominant species in the shallow eutrophic Loosdrecht lakes in The Netherlands, from which it was isolated in 1984. Unlike *Prochloron*, the newly isolated species is filamentous and planktonic. Detailed investigation of its cell structure, composition and physiology is possible as it can easily be grown in a mineral medium.

Batch cultures of the strain were maintained on an orbital shaker at 20°C in a mineral medium⁵ at a photon-flux density of 30 $\mu\text{E m}^{-2} \text{s}^{-1}$ (warm, white fluorescent lamps) and an alternating light/dark regime of 16 h:8 h. The strain was characterized by pigment analysis, electron microscopy and growth response in the presence of antibiotics.

Pigment analysis by HPLC (ref. 6) shows the distinct peak and retention time characteristic of chlorophyll *b* (Fig. 1A). After fractional separation, spectrophotometric analysis confirmed the presence of chlorophyll *b*, with absorption maxima at 454 and 643 nm and an absorbance (A_{454}/A_{643}) ratio of 2.82 (Fig. 1B)⁷.

Chlorophyll *a* contents determined from turbidostat cultures vary between 6 and 36 mg per g dry weight, depending on irradiance level. Preliminary results indicate that the chlorophyll *a/b* ratio is rather constant at photon-flux densities ranging from 4 to 150 $\mu\text{E m}^{-2} \text{s}^{-1}$. The chlorophyll *a/b* ratio varies between 8.1 and 9.0 and is relatively high compared with the ratio commonly found for green algae (2-3)⁸ and for *Prochloron* (4-7)⁹.

Table 1 Pigment composition determined by HPLC

	Dry weight ($\mu\text{g mg}^{-1}$)
<i>a</i> β -Carotene	4.1
<i>b</i> Phaeophytin <i>a</i>	0.8
<i>c</i> Not identified*	ND
<i>d</i> Chlorophyll <i>a'</i>	0.7
<i>e</i> Chlorophyll <i>a</i>	24.0
<i>f</i> Chlorophyll <i>b</i>	3.0
<i>g</i> Zeaxanthin	3.5
<i>h</i> Not identified†	ND
<i>i</i> Not identified†	ND

Letters in left-hand column refer to peaks in the absorbance chromatogram of Fig. 1A. ND, not determined as there is no available standard.

* Probably canthaxanthin; † probably derivatives of zeaxanthin.

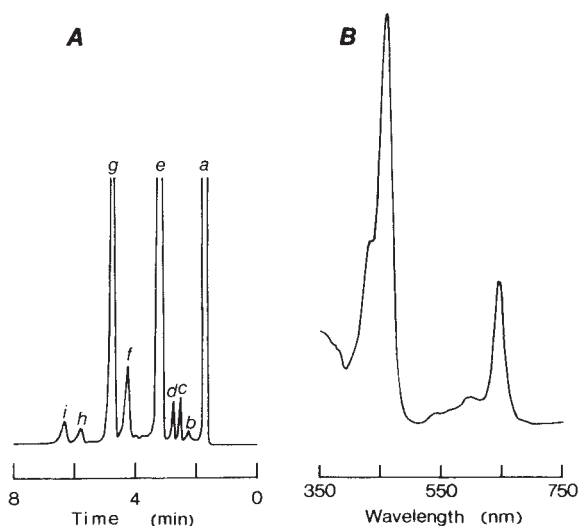


Fig. 1 A, Absorbance (440 nm) chromatogram obtained by HPLC (normal phase, 1,200 p.s.i., semi-preparative column, eluent: 25% acetone in hexane) of an extract of the Loosdrecht isolate. a-i, Peaks of individual pigments listed in Table 1. B, Absorption spectrum of compound f.

No water-soluble pigments could be detected using the extraction technique described by Tandeau de Marsac¹⁰. In attempts to stimulate the formation of phycobilin pigments, cultures were grown in green light or at a low photon-flux density ($4 \mu\text{E m}^{-2} \text{s}^{-1}$), but in neither case were phycobilin pigments found.

The carotenoid composition (Table 1) resembles that of cyanobacteria as no α -carotene and lutein were detected by HPLC (Fig. 1A). Lutein is usually found to be the major carotenoid in green algae, zeaxanthin and β -carotene being less abundant, whereas the latter two pigments are often dominant in cyanobacteria¹¹. Echinenon, a characteristic carotenoid for most cyanobacteria, although not found in *Anacystis nidulans*^{11,12}, is absent in this organism.

Cytology of the cells, including the photosynthetic membranes, was best preserved after pre-fixation in glutaraldehyde and post-fixation in KMnO_4 (ref. 13) (Fig. 2A). Thin sections of filaments fixed by this method indicate that the thylakoid membranes are present only at the periphery of the cells. Phycobilisomes are not present (Fig. 2C) and the photosynthetic membranes are not separated from the cytoplasm by a bounding membrane, that is, chloroplasts are not present. We did not observe other membrane-bound organelles. In cells fixed by glutaraldehyde/ OsO_4 , a nucleoplasmic region surrounded by

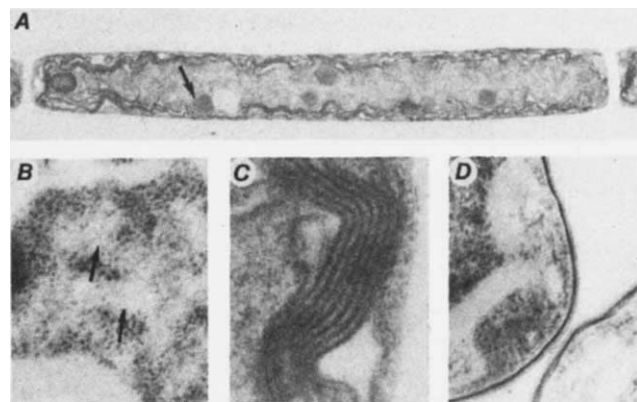


Fig. 2 A, Survey of a cell, fixed in glutaraldehyde/ KMnO_4 , to show overall morphology. The thylakoid membranes are localized close to the cell wall. Arrow, cytoplasmic polyhedral bodies. $\times 11,520$. B-D, Details of cells to show the presence of DNA (arrows) in the cytoplasm (B, glutaraldehyde/ OsO_4 ; $\times 49,920$), the substructure of the thylakoid membranes (C, glutaraldehyde/ KMnO_4 ; $\times 76,320$) and the cell wall (D, glutaraldehyde/ OsO_4 ; $\times 28,800$).

ribosomes can be distinguished in the central region (Fig. 2B). Another characteristic of prokaryotes is the presence of polyhedral bodies (Fig. 2A), which are thought to represent carboxysomes¹⁴.

The cell wall resembles that of cyanobacteria and Gram-negative bacteria (Fig. 2D). The thick electron-dense layer adjacent to the plasmalemma is probably peptidoglycan¹⁵. No mucilaginous sheath was found.

Growth inhibition experiments confirm the prokaryotic nature of the cells. We added cycloheximide (Boehringer), chloramphenicol (Sigma) or cephalosporin (Eli Lilly) to freshly diluted batch cultures of the new organism, of the prokaryote *Oscillatoria agardhii* and of the eukaryote *Scenedesmus protuberans*. Cycloheximide is known to inhibit eukaryotic protein synthesis by blocking the action of 80S ribosomes. Table 2 shows that cycloheximide inhibited growth of *S. protuberans* at a concentration of 1 mg l^{-1} , whereas 20 mg l^{-1} of cycloheximide was only slightly inhibitory to the growth of *O. agardhii* and of the new prochlorophyte. Chloramphenicol inhibits protein synthesis on 70S ribosomes, not only those present in prokaryotes, but also those in chloroplasts and mitochondria of eukaryotes. However, chloramphenicol does not inhibit the growth of eukaryotes if it is applied in low concentrations, as we demonstrated in control experiments with *S. protuberans*, which still grew at a chloramphenicol concentration of 20 mg l^{-1} . For both *O. agardhii* and the new Loosdrecht isolate, growth was inhibited over the whole range of inhibitor concentrations. Cephalosporin acts as an inhibitor of cell-wall synthesis in prokaryotes¹⁶. Table 2 shows that no growth occurred in batch cultures of *O. agardhii* and of the Loosdrecht isolate after addition of cephalosporin. The growth of *S. protuberans* was not affected by cephalosporin.

Thus, the newly isolated organism seems to be a 'normal' prokaryote with respect to its cytoplasmic ultrastructure, cell-wall structure and its response to antibiotics. We have shown that a photosynthetic apparatus consisting of chlorophylls a and b is not strictly restricted to eukaryotic algae and higher plants, and thereby have confirmed the earlier observation of Lewin¹. With regard to structure and pigment composition, the alga is comparable to *Prochloron*.

The discovery of a second prokaryote containing chlorophyll b contributes to the taxonomic and phylogenetic discussion on Prochlorophyta, particularly as the newly isolated species can easily be grown in batch as well as continuous cultures.

Table 2 Inhibition of growth of batch cultures of the Loosdrecht isolate (LI), *O. agardhii* and *S. protuberans*

Antibiotic	Concentration (mg l^{-1})	LI	<i>O. agardhii</i>	<i>S. protuberans</i>
Cycloheximide	1	G		I
Cycloheximide	2	G	G	
Cycloheximide	20	G/I	G/I	
Chloramphenicol	0.2	I		
Chloramphenicol	2	I	I	
Chloramphenicol	20	I	G	
Cephalosporin	3	I		
Cephalosporin	30	I	I	G
Cephalosporin	300	I		

I, inhibition; G, growth.

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Serial and parallel processing of visual feature conjunctions

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Treisman and others¹⁻³ have reported that the visual search for a target distinguished along a single stimulus dimension (for example, colour or shape) is conducted in parallel, whereas the search for an item defined by the conjunction of two stimulus dimensions is conducted serially. For a single dimension the target 'pops out' and the search time is independent of the number of irrelevant items in the set. For conjunctions, the search time increases as the set becomes larger. Thus, it seems that the visual system is incapable of conducting a parallel search over two stimulus dimensions simultaneously. Here we extend this conclusion for the conjunction of motion and colour, showing that it requires a serial search. We also report two exceptions: if one of the dimensions in a conjunctive search is stereoscopic disparity, a second dimension of either colour or motion can be searched in parallel.

Visual search experiments were conducted with the aid of a Commodore 64 microcomputer and colour television monitor. Two types of experiment were performed, those where the observer's task was to see the unique target which differed from all others in one dimension only (simple); and those where the observer had to find the unique target which was defined by two dimensions (conjunction). The stimulus consisted of a variable size array containing either 15, 25 or 35 targets (see Fig. 1). Each of these targets was a random pattern containing 16 picture elements or pixels. Depending on the experiment (see Fig. 2), the targets were clearly distinguishable either in terms of colour (red versus blue), motion (up versus down) or stereoscopic depth (either in the fixation plane or in front). In the case of stimulus motion, each target formed a rectangular 'aperture' behind which a continuous array of random dots could be moved, either up or down.

The observer was aware of the particular dimension(s) to be searched before the beginning of each block of trials; the task was to press a button as soon as the target was seen. To eliminate false-positives, the array remained visible for an additional 800 ms after the response. This enabled the observer to scrutinize the display and to verify whether his identification was correct.

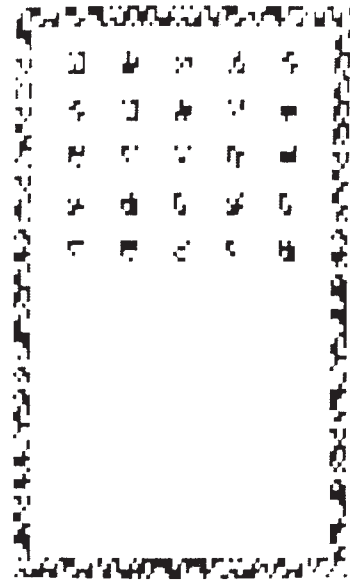


Fig. 1 The stimulus array contained either 35 targets (7 rows), 25 targets (5 rows) or 15 targets (3 rows) and was bordered with a frame to ensure stereoscopic fusion in the binocular experiments. For observer K.N., the stimuli had the following parameters: target size $0.4 \times 0.5^\circ$, frame size $7.5 \times 13^\circ$, vertical spacing of targets (1.0°), horizontal spacing of targets (1.2°), disparity 20 arc min, velocity $3.75^\circ \text{ s}^{-1}$. All stimulus parameters were 25% smaller for observer J.S.

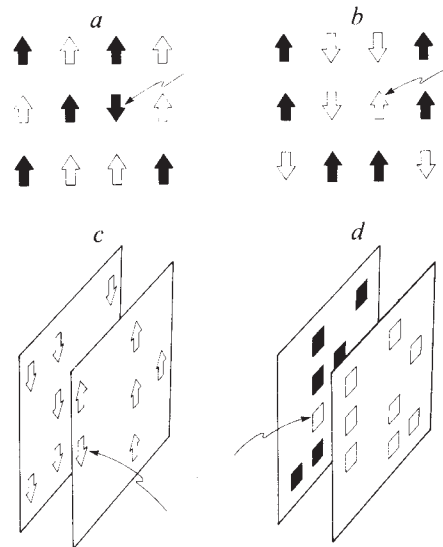
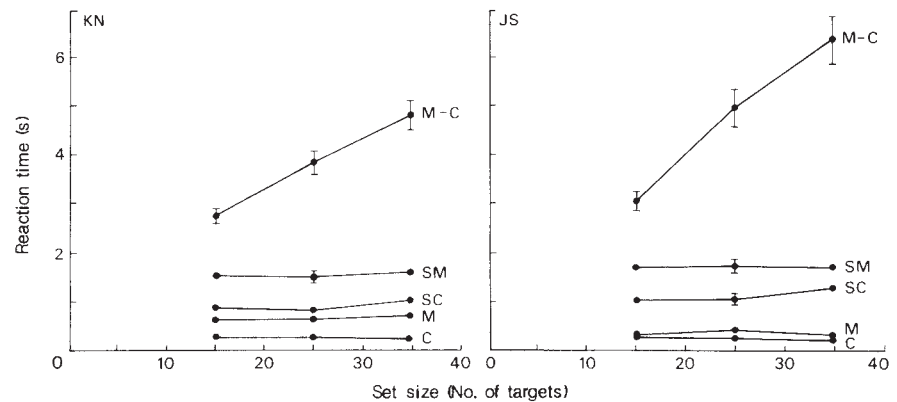


Fig. 2 *a*, Schematic description of the simple one-dimensional search for a discrepant motion. The observer must find the single target that has the anomalous motion with respect to all motions, targets are randomly coloured. *b*, Conjunctive search for colour and motion. Blue distractors move down, red distractors move up. The target breaks this pattern and is either a blue moving up or a red moving down. *c*, Conjunctive search for stereo and motion. All distracting targets in the front plane are moving up and those in the back plane are moving down. Relevant target either is in front and moves down or in back and moving up. *d*, Conjunctive search for stereo and colour. Distracting targets in the front plane are blue and those in the back plane are red. Relevant target is either red in front or blue in back. Open symbols, blue colours; filled symbols, red colours. Target to be found is marked by small pointer.

The data associated with mistakes were discarded.

Each set of experiments had the same overall design and differed only in the choice of stimulus dimensions to be searched. In any given experiment, two stimulus dimensions were used: motion-and-colour (M-C); stereo-and-motion (SM), or stereo-

Fig. 3 Reaction times plotted as a function of the number of elements in the set to be searched. Results from two classes of experiments are shown. Conjunction: motion-colour (M-C), stereo-motion (SM), stereo-colour (SC). Simple: colour (C), motion (M), stereo (S). Note that for the conjunctive search of M-C, the anomalous target becomes more difficult to find as set size increases. This is not the case for the conjunction of SM or SC. All plotted reaction times represent the mean of at least 40 separate trials, preceded by at least 40 unscored practice trials.



and-colour (SC). For the simple search, the observer was required to find the target that was distinguished by a unique feature along a single dimension. In the case of motion (M) it was the target which moved in the opposite direction to all others, with colour being randomly assigned to all targets on an equiprobable basis (Fig. 2a). For the case of stereo (S), it was the single target which had a different binocular disparity from all other targets, with colour being randomly assigned to the targets on an equiprobable basis. For the case of colour (C), it was the single target which had the odd colour with the different directions of motion being randomly assigned.

Our first set of experiments extend and confirm previous work^{1,2} showing that the search for a single feature is conducted in parallel. Reaction times are short and remain constant as the set size increases. Lower reaction time functions designated C, S and M (Fig. 3) refer to the simple search for colour, stereo and motion, respectively. All indicate that for the single-dimensional search, the processing is pre-attentive and effortless.

Conjunctive search, however, has been shown to require serial processing. We therefore expected that a search requiring the conjunction of motion and colour would also be conducted in serial. The irrelevant targets (distractors) for this experiment were coloured either red or blue on a random equiprobable basis, with upwards motion linked to the red distractors and downwards motion linked to the blue. The relevant target broke this pattern, so it could be either a red moving down or a blue moving up (Fig. 2b). Compared with the simple search, observers found this task to be extremely difficult—they were unable to perform a parallel search for the anomalous motion over a given colour. Instead, they resorted to searching very small portions of the display, almost target by target. These subjective impressions were amply supported by the reaction time functions. The M-C function rose steeply (>100 ms per item) for increasing set sizes, confirming our expectation that the conjunctive search for motion-and-colour is indeed serial (Fig. 3).

In our final set of experiments we introduced the conjunction of stereoscopic disparity with either motion or colour. We generated two separate images on the television monitor and used crossed-polaroid filters to obtain the necessary dichoptic separation to produce the stereograms. The fixation plane was defined by a rectangular border which enclosed the targets and was always visible between trials. The individual targets were randomly assigned to either depth plane with equal probability. For the case of stereo-and-motion, the distractors in the front plane were always moving up and those of the back plane were always moving down. The target to be found was either moving down in the front plane or moving up in the back plane (Fig. 2c). For the case of stereo-and-colour, the distractors in the front plane were always blue and those in the back plane were always red. The target to be found was either a red in the front plane or a blue in the back plane (Fig. 2d).

In comparison with the case of motion-and-colour, these conjunctive tasks were qualitatively different and much easier. The observer had the distinct impression that each plane could

be searched almost effortlessly, in turn. Correspondingly, the reaction time functions for each of these searches are constant over set size, never rising to the high values of reaction time which we found for the M-C search. This can be seen in the functions for SM and SC in Fig. 3. It seems that the visual system can perform a parallel search in one depth plane without interference from target-like distractors in another depth plane.

Our data suggest that the visual system can sequester processing or restrict attention in the spatial dimension (see refs 4, 5) but not to other visual dimensions such as colour and motion. Treisman² found that observers can attend to different two-dimensional loci in a complex visual scene, serially, yet perform a search within a given two-dimensional region, in parallel. We show that an analogous process occurs with different stereo-depth planes. Each depth plane can be processed in turn, allowing a parallel search within each plane.

We speculate that retinal disparity in addition to retinal locus has priority when compared with other visual stimulus dimensions; perhaps they constitute a set of primary indices to which the other visual attributes are linked⁶. As such, the results can be related to the anatomical organization of visual cortical projection areas, suggesting a segregation and duplication of visual features according to disparity. Thus, visual features of motion and colour at one disparity could be separately encoded and duplicated at other disparities. This notion of conjunctive parallel processing for dimensions linked to disparity finds a neurophysiological correlate in visual cortex area MT for the case of stereo motion⁶ where single cells are selectively tuned to both direction and disparity. The existence of comparable cells selective for both colour and disparity has yet to be reported.

Conversely, the serial search associated with the conjunction of motion and colour seems to preclude the existence of single units selectively tuned to both motion and colour, consistent with recent evidence regarding the functional segregation of colour and motion information within cortical area V2 (refs 7–10) as well as their separate corresponding target destinations in V4 and MT. Cells in V4 are sensitive to wavelength differences but not to the direction of motion¹¹, whereas MT cells are sensitive to motion and not to wavelength¹².

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Homing behaviour of axons in the embryonic vertebrate brain

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In embryonic nervous systems, growing axons must often travel long distances through diverse extracellular terrains to reach their postsynaptic partners. In most embryos, axons grow to their appropriate targets along particular tracts or nerves, as though they were following guidance cues confined to specific pathways¹⁻³. For example, in all vertebrates, axons from the retina invariably grow to the tectum along the well-defined optic tract⁴⁻⁶. Yet, transplant experiments demonstrate that retinal axons make tectal

projections even though they enter the brain at locations which are distinctly off the optic tract⁷⁻¹¹. Only recently has it become possible to label discreet growing projections in the embryonic vertebrate brain¹². Thus, it is not yet known whether displaced retinal axons grow directly towards the tectum or find it accidentally, through random extension. To resolve this question, pioneering axons from normal and transplanted eyes in embryonic *Xenopus* were labelled using a short-survival horseradish peroxidase (HRP) method⁴, and their orientation during growth was quantitatively assessed. The finding that the ectopic fibres head towards their distant targets implies that guidance cues are not restricted to specific pathways but are distributed throughout the embryonic brain. The significance of this result is discussed with respect to the ontogeny and evolution of the visual pathway.

Xenopus embryos at ~24 h post-fertilization (PF) (Nieuwkoop and Faber¹³ (NF) stage 24) were prepared for surgery in modified Ringer's solution with anaesthetic (0.01% tricaine) and antibiotics (25 µg ml⁻¹ gentamycin). Eye primor-

Fig. 1 *a-c*, Whole-mounts of normal embryonic *Xenopus* brains with labelled optic pathways, viewed laterally (*a* at stage 33/34, *c* at stage 35/36). *b*, Growth cones from *a* at higher magnification. *d-h*, Camera lucida drawings of brains fixed at stages 33/34-40, during which time the retinotectal projection normally develops. Dotted lines in *c* and in most of the camera lucida drawings show the three major subdivisions of the brain: Fo, forebrain; Mi, midbrain; Hi, hindbrain (see *g*). *h*, The protocol for estimating the coordinates of the central tectal neuropil (Tn): a line was drawn from the area of the chiasm (*y*) to the midpoint of the dorsal midbrain (*y'*); at right angles to this, a second line was constructed from the boundary between the midbrain and hindbrain (*x*) to the dorsal-most boundary between the forebrain and midbrain (*x'*). These lines were normalized and served as axes in a cartesian coordinate system. Next, in this stage-40 embryo, a star was placed on the area of most dense midbrain innervation, the tectal neuropil (as determined from visual inspection), and the coordinate pair that best represented this estimated tectal centre was recorded. In this case the respective (*x*, *y*) values were (0.57, 0.70). This procedure was repeated for seven animals at stages 39 and 40 and yielded an average result of (0.55 ± 0.06, 0.73 ± 0.03; mean ± s.d.). These average values were used as the location of the estimated tectal neuropil (ETn) in all experimental animals (see Figs 2, 3) and in normal embryos at stages when retinal axons had not yet reached the target, as shown in *a* and *d*. This quantitative method of estimating the location of the tectal neuropil was necessary because there are no visible independent markers of this structure at early stages. The high reproducibility of the coordinate values in control animals indicates that this method gives a very reasonable, if imprecise, location of the target area. Ch, chiasm; Te, telencephalon; Di, diencephalon; Tcm, tectal cell mass; Pi, pineal; Hy, hypothalamus.

Methods. To label embryonic retinal projections, HRP was injected locally using the following procedure. The cornea and lens were removed from anaesthetized and restrained animals and a glass pipette with a terminal bore of ~30 µm, partially filled with a solution of 20% HRP in 2% lysolecithin, was inserted into the lens cavity. By application of gentle pressure at the back of the pipette, the label was forced onto the vitreal surface of the retinal cup. After about 5 min of superfusion of the retina, the embryo was allowed to recover for 15-30 min, after which it was again anaesthetized and then immersed in ice-cold fixative (1.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer). After 1 h of fixation, the embryo was rinsed overnight in buffer, and the brain was dissected out and treated histochemically with diaminobenzidine to allow visualization of HRP. The brains were then dehydrated and mounted in methyl salicylate or Fluoromount for examination as whole-mounts.

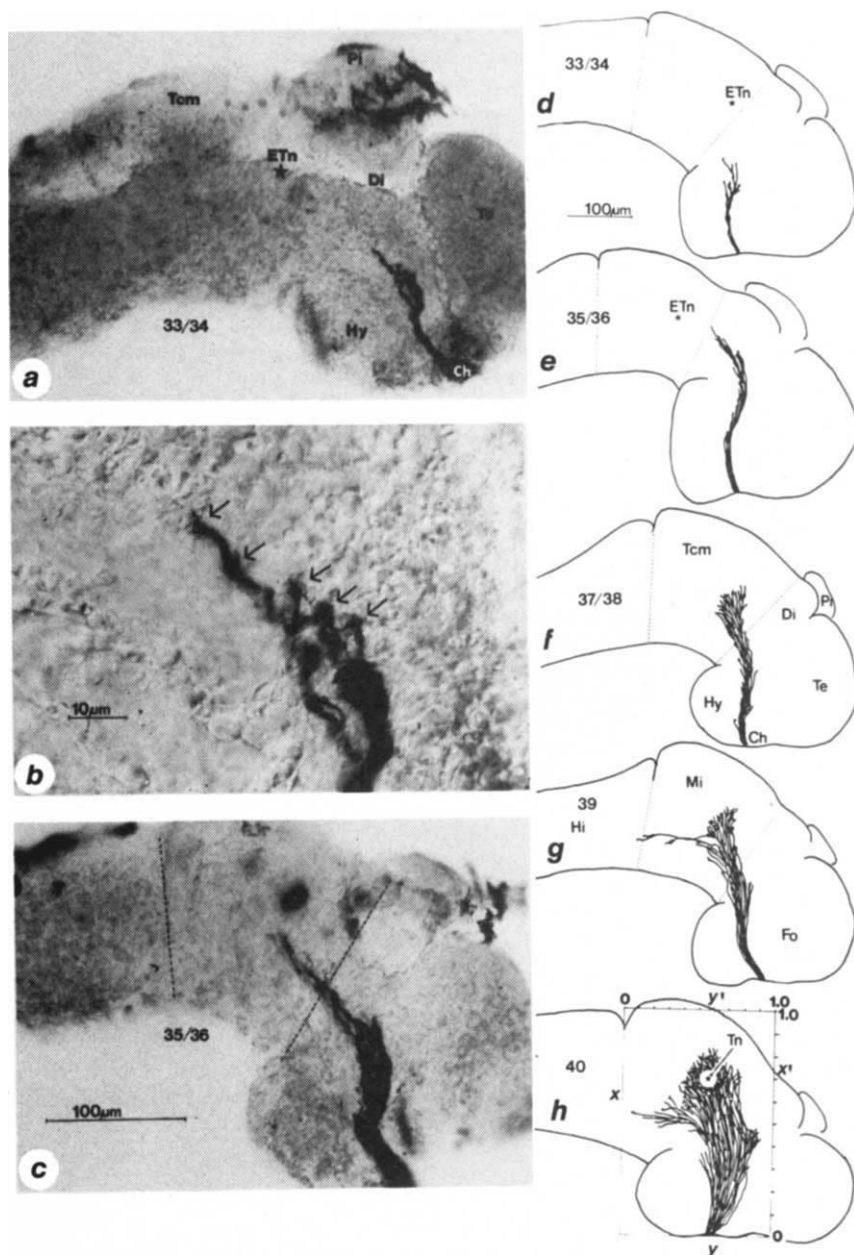
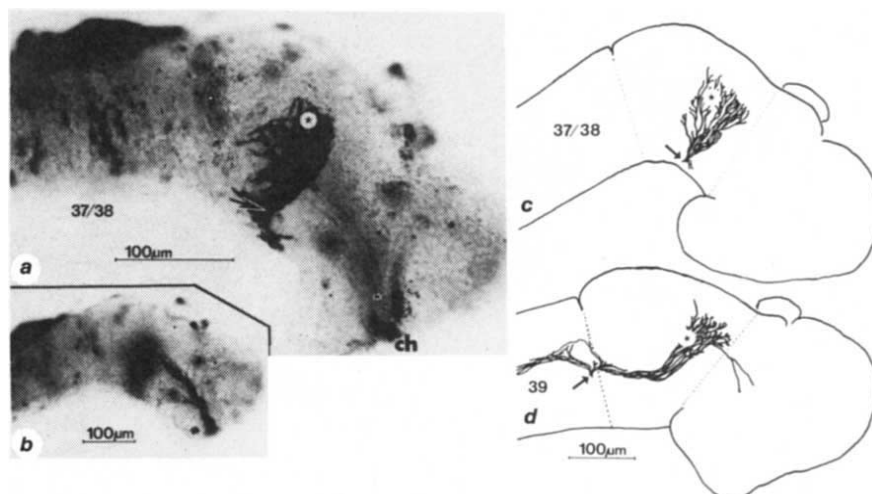


Fig. 2 Ectopic projections at the time of fibre arrival in the tectum in three animals at stages 37/38 and 39. *a, b*, Photograph of the same stage-37/38 whole-mount (arrow marks entry point); *c, d*, camera lucida drawings of two other cases. Stars indicate the estimated tectal neuropil (see Fig. 1). Note that the ectopic axons migrate to this location. *b*, The control pathway in same animal as *a*. The brain was photographed from the other side and the print made from the reversed negative; this gives the impression that we are focusing through the brain. Note that in *b* the shadow of the ectopic pathway is still visible and can be compared directly with the normal optic pathway. The only overlap is at the tectal neuropil, indicating that the ectopic fibres have reached the normal target by a novel pathway. The entry points (arrows) of the transplanted optic fibres in both *a* and *c* are within the midbrain, and the projection is almost entirely to the ipsilateral tectum. In *d*, the transplanted nerve entry point is near the junction between midbrain and hindbrain and, in this case, some fibres travel down the spinal cord while the rest travel to the tectum.



dia, including epidermis and optic stalk, were excised and inserted into an excavation in the other side of the head of the same embryo. Usually the eye contralateral to the excised one was left in place to ensure that the optic stalk of the transplant healed into the brain at a separate location from that of the control eye. In these cases the embryo developed with two eyes on the same side of its head, a sort of artificial flounder. In some instances, the contralateral eye was removed because it obstructed a desired ectopic transplant site. After the transplant had healed into place (~10–30 min) the embryo was removed to a 10% Holtfretter's¹⁴ solution where it remained until the stages at which the normal retinal axons invaded the brain and grew towards the tectum (NF stages 33/34–40; 44–66 h PF). At these stages the retinotectal projections of both normal and experimental animals were labelled with HRP (see Fig. 1 legend). The orientation of the displaced axons with respect to their target was assessed using a quantitative method for estimating the location of the tectal neuropil (see Fig. 1 legend), combined with the construction of an orientation index (see Table 2 for details).

The development of the retinotectal projections in whole-mounts of the *Xenopus* brain was examined in 46 normal and 25 experimental embryos. The normal cases (Fig. 1) showed that by stage 33/34 (44 h PF, Fig. 1*a, d*), leading axons are usually in the ventral or mid-diencephalon and climbing towards the tectum. At stage 35/36 (50 h PF, Fig. 1*c, e*) the axons arrive at the dorsal diencephalon, and the first axons reach the tectum at about stage 37/38 (53 h PF, Fig. 1*f*). During stages 39 (56 h PF) and 40 (66 h PF, Fig. 1*g, h*) the innervation of the tectum becomes more dense^{12,15}. When the pathway is being forged, the pioneering axons are capped with enlarged growth cones (Fig. 1*b*). Often many such axons, slightly separated from each other, were seen in a single preparation, suggesting that each of the early growing axons may be navigating to the tectum independently. This is consistent with the recent demonstration that the first axons to travel the pathway from eye to tectum, the ones originating from the dorsal retina, do not serve as necessary pioneers for the later-growing axons from the ventral retina^{15,16}. The notion of independent navigation is also consistent with the finding for ectopic axons presented below.

To determine whether ectopic axons grow directly to the tectum and innervate it, 14 of the experimental embryos were examined at stages 37/38–40, by which time normal optic axons would have just reached the tectum. To determine whether these ectopic axons orient towards the target from a distance, the other 11 embryos were fixed at stages 33/34–35/36, before optic axons normally reach the tectum.

All 14 of the transplants examined at stages 37/38–40 sent major projections directly to the ipsilateral tectum, where most of the fibres ended in a dense arborization (Fig. 2). In a few cases, where the ectopic entry point into the brain was near the normal optic tract, an additional bundle of fibres was seen travelling through the chiasm region and towards the contralateral tectum, the side of origin of the transplant. When the transplanted optic nerve penetrated the brain near the medulla, some fibres travelled down the spinal cord (Fig. 2*d*). These different secondary projections were expected, as previous studies have shown that ectopic optic nerves entering the diencephalon often send projections to both the ipsilateral and contralateral tectum¹⁷, and optic fibres entering the hindbrain in frogs and salamanders travel down the spinal cord in a dorsolateral tract¹⁸ (Table 1). A significant feature of the cases reported here is that the ectopic projections were examined at the earliest stages that fibres could have innervated the tectum even in normal animals. The fact that there were no unusual projections implies that these fibres did not head out in arbitrary directions at early stages. Rather, almost all of the ectopic fibres appeared to have advanced directly towards their targets. The present results also show that ectopic axons did not first seek the path where the optic tract would normally lie, and from there navigate on the normal pathway; they appeared instead to run along nearly straight lines between the entry point and

Table 1 Projections of ectopic optic nerve fibres at the time of reaching the tectum (stage 37/38–40 embryos)

Entry point of optic nerve	Site of projection			Other
	Ipsilateral tectum and chiasm	Ipsilateral tectum only	Ipsilateral tectum and spinal cord	
Forebrain (n = 3)	3	—	—	—
Midbrain (n = 6)	—	4	2	—
Hindbrain* (n = 5)	—	1	4	—

All transplants with central projections sent fibres to the ipsilateral tectum. In addition, those cases in which the ectopic entry point was in the forebrain sent an additional fascicle of fibres into the optic chiasm whereas those that entered the rostral hindbrain sent fibres down the spinal cord as well as to the tectum (see Fig. 2*d*).

* Entries in this row were all very near to the junction between midbrain and hindbrain.

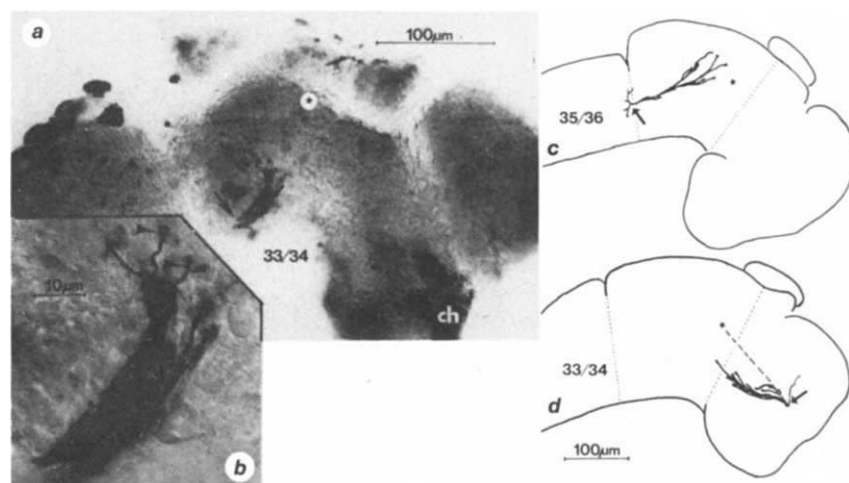


Fig. 3 Ectopic projections before the fibres have reached the tectum in three animals (stage 33/34–35/36). *a*, Whole-mount of a stage-33/34 animal viewed laterally. Arrows mark entry points and stars indicate the estimated tectal neuropils (calculated as described in Fig. 1 legend). *b*, A higher magnification of growth cones (arrowheads) from *a*. *c*, *d*, Camera lucida drawings of two other cases. Note that the fibres are heading directly for their estimated targets. In *d* the broken line constructed between the entry point and the estimated tectal neuropil serves as the 0° line for the polar orientation graph shown in Fig. 4a.

the target, often overlapping with the normal pathway only at the tectum (Fig. 2b).

To examine the orientation of displaced axons at stages before normal optic fibres would have reached the tectum, experimental projections were labelled in even younger embryos. Ectopic axons proved similar to normal ones in that at stages NF 33/34 and NF 35/36, though they had not yet reached the tectum, they were growing towards it. In the experimental embryos, ectopic fibres oriented towards the ipsilateral tectum (Fig. 3). A quantitative measure of fibre orientation (the orientation index, *OI*; see Table 2 for details) was obtained by considering the abnormal entry point into the brain as the origin in a polar coordinate system, with a line directly to the estimated tectal neuropil serving as the 0° angle (Fig. 3d). When the growth cone-tipped fibres (Fig. 3b) were traced by camera lucida techniques onto

such polar graphs and their orientations analysed statistically, it became clear that these axons were nonrandomly heading for the tectum (Fig. 4, Table 2).

The finding that displaced axons orient towards their central target suggests that guidance cues in the vertebrate brain are not restricted to particular pathways, but appear to be broadly distributed, so that axons accidentally veering off course can perseveringly 'home-in' on their proper targets. This implies a resilience in the development of neural connections, and can explain both transplant results and sizeable variations in the projections of normal optic axons to the tectum (Figs 1d, e and 4e, f). A distributed guidance mechanism may also have evolutionary significance. For example, if variants occur that slightly alter the placement of the eye primordium, as appears to have happened throughout vertebrate phylogenesis, this

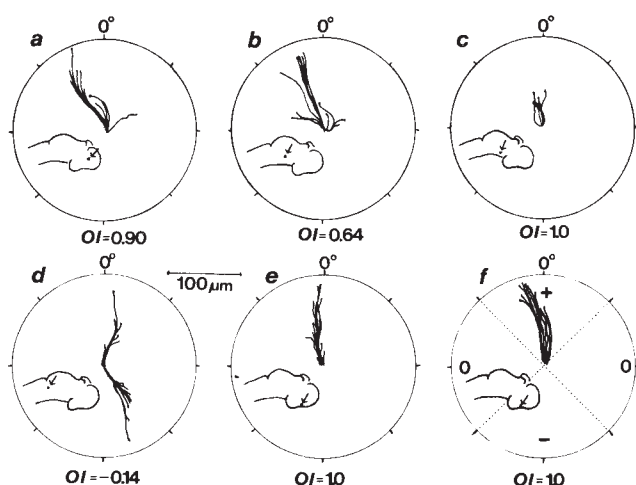


Fig. 4 Orientation graphs of ectopic (*a–d*) and normal (*e, f*) axons at stages 33/34 and 35/36. Using the ectopic entry point (or in normal animals the chiasm) as the origin, camera lucida drawings of the fibres were made on polar coordinates with 0° indicated as the direct line between the entry point and the estimated tectal neuropil. *a*, Same animal as in Fig. 3d; the rest are different cases. In *f*, dotted lines divide the circle into four quadrants (+, 0, – and 0). That most of the fibres in these cases are restricted to the (+) quadrant (see also Table 2) indicates that normal and ectopic retinal fibres seem to orient towards their targets from a distance. In case *d* a major group of fibres is also heading into the (–) quadrant; the entry point in this case is near the midbrain–hindbrain junction and these fibres are running towards the spinal cord. The schematic insets show the approximate entry points of the fibres into the central nervous system for each case. *OI*, the orientation index, which describes a weighted proportion of fibres heading towards their targets (see Table 2 for definition of *OI*).

Table 2 Orientation of optic nerve fibres before they have reached the tectum (stage 33/34 and 35/36 embryos)

Entry point of optic nerve	Orientation index
Chiasm (normal animals, <i>n</i> = 10)	1.0 ± 0.0
All ectopic entry points (<i>n</i> = 11)	0.41 ± 0.18
Ectopic entry points in forebrain and midbrain only (<i>n</i> = 8)	0.69 ± 0.16

The orientation of projections from both normal and ectopic retinal fibres was analysed by assigning an orientation index (*OI*) to each labelled projection. This was done using the polar graphs shown in Fig. 4. Each individual fibre receives a score of +1 if it enters the (+) sector (towards the target), 0 if it enters either of the (0) sectors (neither towards nor away from the target), and –1 if it enters the (–) quadrant (away from the target). The total *OI* for an animal is taken as the sum of scores for the individual fibres divided by the total number of fibres counted. Thus, an *OI* of 1.0 indicates perfect orientation found in normal animals; a score of 0 would indicate no preference, and negative values would indicate that fibres tend to orient away from the tectum. The average score of 0.4 for all the ectopic projections means that optic fibres entering the brain anywhere (within the scope of those represented here) are more likely to orient towards the tectum [(+) quadrant] than run in any other direction [(0) or (–) quadrants]. If one considers only optic fibres that enter the forebrain or midbrain (eliminating all spinal cord projections) the *OI* rises to near 0.7. The reason that the *OI* is higher when hindbrain entry points are excluded is that a number of fibres entering in the hindbrain may be attracted to the alternative target, the spinal cord. Thus, for example, the *OI* of –0.14 in Fig. 4d belies the obvious orientation of a bundle of the fibres heading towards the tectum in this case. A χ^2 analysis, used to test the random orientation of fibres (that is, 25% to each quadrant) showed that this possibility could be excluded at the $P < 0.005$ level. Similarly, a one-tailed student's *t*-test showed that the percentage of fibres found in the (+) quadrant was significantly greater than the 25% random expectation ($P < 0.005$). Values shown are mean ± s.e.m.

would not jeopardize the formation of a central visual pathway. Such 'long-range' or distributed guidance mechanisms may also account for the remarkable ability of various brain transplants in embryonic or neonatal mammals to make functional connections¹⁹. There are, however, limits to the range of these guidance cues, for if displaced by too great a distance, ectopic axons may fail to project to their normal targets. For example, when the optic nerve enters the medulla or cord, spinal rather than tectal projections are often made^{17,18}. The present results suggest that the cues for appropriate optic fibre guidance must be distributed at least throughout the diencephalon and mesencephalon. The molecular nature of these guidance cues is unknown. One might suspect a chemotactic mechanism^{20,21} with the tectum acting as a source of a diffusible attractant. *In vitro* studies have demonstrated trophic effects of soluble factors from the tectum on retinal ganglion cell survival and neurite elongation, yet there has been no *in vitro* demonstration of chemotactic guidance in this system^{4,22-24}. This raises the possibility that growth cones can detect cell surface molecules which signal positional information throughout the entire brain^{25,26}.

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Pathway selection by growth cones of identified motoneurons in live zebra fish embryos

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How is the adult pattern of connections between motoneurons and the muscles that they innervate established during vertebrate development? Populations of motoneurons are thought to follow one of two patterns of development: (1) motor axons initially follow stereotyped pathways¹ and project to appropriate regions of the developing muscle²⁻⁴ or (2) motor axons initially project to some regions that are incorrect, the inappropriate projections being eliminated subsequently⁵⁻⁹. Here we observed individually identified motoneurons in live zebra fish embryos as they formed growth cones and as their growth cones navigated towards their targets. We report that from axogenesis, each motor axon followed a stereotyped pathway and projected only to the specific region of the muscle appropriate for its adult function¹⁰. In addition, the

peripheral arbor established by each motoneuron was restricted to a stereotyped region of its own segment and did not overlap with the peripheral arbor of the other motoneurons in that segment. We conclude that the highly stereotyped pattern of innervation seen in the adult is due to initial selection of the appropriate pathway, rather than elimination of incorrect projections.

Zebra fish embryos offer several advantages as a system in which to study neuronal development. The embryos are optically clear, and development occurs rapidly, so we can follow a neurone in a single individual from the time it is first recognizable until its axon reaches its targets. Here we observed the growth of live motoneurons in trunk segments 6-15 (Fig. 1a, b) with Nomarski (Fig. 1c) or fluorescence (Fig. 1d, e) optics.

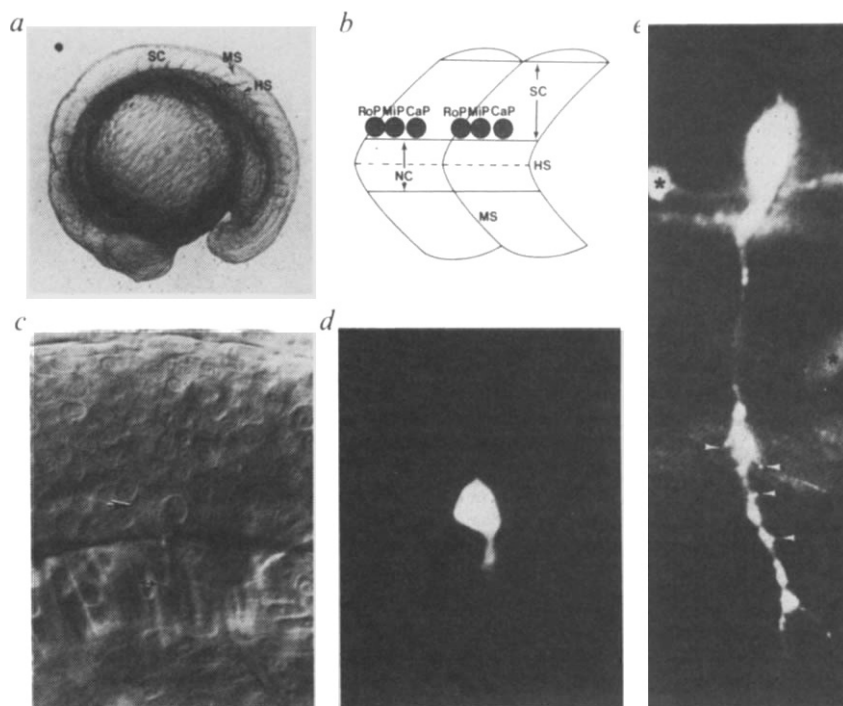
Initially there were three motoneurons on each side of the spinal cord in each segment (Fig. 1b). These cells were recognizable as primary motoneurons^{11,12} even prior to axogenesis by their large diameter ($9.8 \pm 0.9 \mu\text{m}$ (mean $\pm$ s.d.); 10 cells, 3 animals) and characteristic ventro-lateral positions within the spinal cord. Each primary motoneuron occupied a specific location in the spinal cord, and we have named them according to this feature (caudal primary, CaP; middle primary, MiP; rostral primary, RoP) (Fig. 1b).

Two independent lines of evidence show that the CaP growth cone in each segment leaves the spinal cord before the growth cones of the other primary motoneurons, thus acting as a 'pioneer' in establishing the ventral root. First, using Nomarski optics (Fig. 1c), we found that the earliest growth cone we could observe in each segment was from CaP (50 segments; 19 animals). Second, in four animals, two or all three of the primary motoneurons within a single segment were fluorescently labelled; in all of these cases the CaP growth cone left the spinal cord first. Although the MiP and RoP growth cones consistently left the spinal cord later than the growth cone of the CaP motoneuron in the same segment, our results did not allow us to determine whether the CaP always initiates an axon before MiP and RoP or whether the CaP growth cone leaves the spinal cord first because it has the shortest distance to grow.

Each primary motor axon follows a stereotyped pathway (Fig. 2) characteristic of its cell type. Each CaP axon (Fig. 2a) had a prominent growth cone that grew directly ventrally, along the medial surface of the muscle. At the region of the horizontal septum the CaP growth cone typically paused for about an hour ($0.9 \pm 0.7 \text{ h}$; $n = 7$). When the growth cone continued ventrally, a prominent varicosity remained at the region of the horizontal septum. The CaP growth cone also typically paused and formed varicosities at the bottom of the notochord and at intervals along the length of the growing axon. Branches formed later at some of these varicosities and grew along the medial surface of the muscle.

In contrast, the MiP growth cone (Fig. 2b) grew caudally within the spinal cord, exited at the ventral root, and followed the pathway established by the CaP axon. In segments in which both CaP and MiP motoneurons were labelled ($n = 4$), the two axons appeared to fasciculate. Like CaP growth cones, MiP growth cones grew along the medial surface of the muscle to the region of the horizontal septum. MiP growth cones also typically paused for about 30 min ($0.6 \pm 0.5 \text{ h}$; $n = 8$) at the horizontal septum. Unlike CaP growth cones, however, MiP growth cones did not cross the horizontal septum. Instead, each growth cone sprouted a branch that grew caudally and dorsally, entering the dorsal portion of the segment. MiP axons typically (8 out of 10) retracted their ventral branches as they grew dorsally, although some cells (the remaining 2 out of 10) retained the ventral branch for up to 48 h post-fertilization (PF). RoP growth cones (Fig. 2c) initially followed the pathway described for MiP growth cones. RoP growth cones grew caudally within the spinal cord, exited at the ventral root, and grew directly to the region of the horizontal septum. From this point, they followed a third pathway, growing laterally within the horizontal

Fig. 1 *a*, Zebra fish embryo at 18 h post-fertilization (PF). The axial musculature is segmented, and the segments are separated by transversely oriented myosepta (MS), and divided into dorsal and ventral regions by the horizontal septum (HS). The chevron-shaped segments are transparent and thus the spinal cord (SC) and the notochord (NC) can be visualized directly. We confined our observations to motoneurons in segments 6–15. In this figure rostral is to the left, and dorsal to the top; all other figures are of the same orientation. Scale bar, 225 μ m. *b*, A drawing of two adjacent segments (abbreviations as in *a*). The solid circles represent the positions of the three primary motoneurons in each segment. Caudal primary (CaP) is located in the middle of the segment, at the level of the ventral root; rostral primary (RoP) is located at the rostral end of the segment; and middle primary (MiP) is located between CaP and RoP. Scale bar 33 μ m. *c*, Nomarski photomicrograph of a CaP motoneurone (large arrow) with a growth cone (small arrow) in a live embryo at 20.5 h PF. Scale bar 10 μ m. *d*, Fluorescence photomicrograph of a living labelled CaP motoneurone (20.5 h PF). Although early growth cones can readily be observed with Nomarski optics, this technique is inadequate for later observations because the development of striations in the muscle fibres obscures axons. Thus, we have also labelled neurones with fluorescent dyes to observe axons and watch their development in live embryos. To label developing primary motoneurons, we injected their early precursor cells with fluorescent dye (fluorescein or rhodamine) conjugated to dextran^{19–21}. We injected single blastomeres in developing blastulae at the 32–512-cell stage; as the cells continued to divide, the label was passed on to all of the progeny of the injected blastomere. The descendants of the injected blastomere scattered during gastrulation²¹, so that individual labelled motoneurons were observed at later developmental stages relatively unobscured by other labelled cells. To observe the development of the motoneurons without causing photodamage²², we used low levels of epifluorescence, monitored the cells with a silicon-intensified-target camera (SIT camera, GE 57)^{23,24} and recorded the image on videotape (VHS) for later analysis. The CaP motoneurone shown was photographed from the face of the video monitor. Scale bar, 10 μ m. *e*, CaP motoneurone in a live embryo at 27 h PF (segment 11). The fluorescence optics showed side branches (arrowheads) and varicosities which were present along the length of the axon at these later developmental times. The body of the main growth cone was also clearly visible although filopodia were usually not resolved. In this cell the growth cone bifurcated shortly before the photograph was taken. The asterisks mark fluorescently labelled cells that were out of focus on the other side of the embryo. Scale bar, 10 μ m.



septum, and forming horizontal branches that extended from the medial surface of the muscle to the lateral surface.

The peripheral arbor of each primary motoneurone is characteristic of its cell type, being restricted to a stereotyped region of the ipsilateral muscle of its own segment. The same three types of motoneurons have been observed in larvae and adults^{12,13}. The branches of CaP motoneurons were confined within the ventral musculature (Fig. 3*a*) in all but a single case (64 out of 65 motoneurons examined until 48 h PF; the remaining CaP motoneurone had a small branch extending into an adjacent segment). In contrast, the branches of MiP motoneurons (Fig. 3*b*) were confined to the dorsal musculature of their own segment (all of 15 motoneurons; 48 h PF). The branches of RoP motoneurons (Fig. 3*c*) also were confined to their own segment, but were restricted to a unique region flanking the horizontal septum (all of 4 motoneurons; 48 h PF).

What factors could be responsible for confining the growth cones of the primary motoneurons within their own segments? One possibility is that the myosepta are barriers to axonal growth, but this seems unlikely as a later-developing class of motoneurons, the secondary motoneurons, have axons that cross these myosepta^{10,13}.

A rostro-caudal gradient in the time of axonal outgrowth within a class of motoneurons might be another possible determinant of segmental specificity; a motoneurone from a caudal segment could not innervate muscle in a more rostral segment, because that muscle would already be innervated. However, two independent observations fail to support this notion. First, in

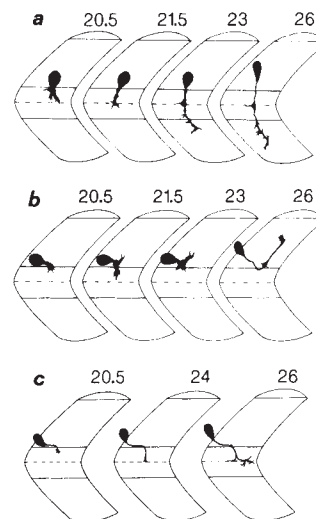


Fig. 2 The axons of the three primary motoneurons of each segment follow divergent pathways. Drawings from the face of the video monitor of fluorescent motoneurons from live animals at the indicated developmental stages (h PF) superimposed on outlines of the segments. *a*, A single CaP motoneurone at four different times; *b*, a single MiP motoneurone at four different times; *c*, a single RoP motoneurone at three different times. (See Fig. 3 for three-dimensional drawings of motoneurons at a later developmental stage.)

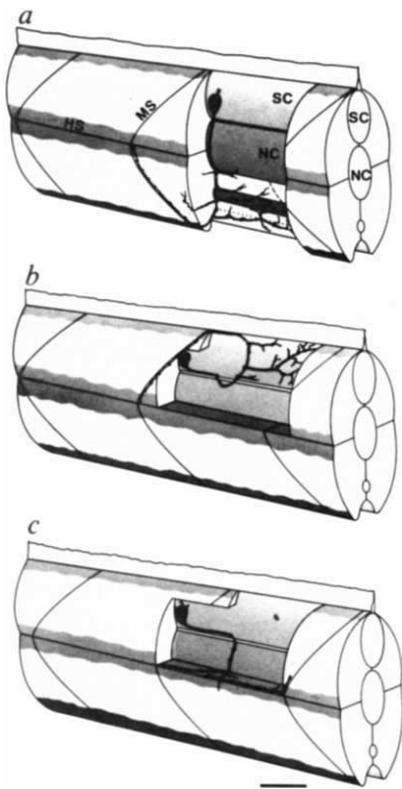


Fig. 3 Each of the three primary motoneurones in a segment has a unique peripheral arbor, as shown by three-dimensional drawings of the arbors of the primary motoneurones at 48 h PF. A portion of the muscle overlying the spinal cord (SC) and notochord (NC) in each part of the figure has been omitted, to show the underlying pattern of the axonal branches. Each motoneurone projects exclusively to muscle fibres within its own segment, and to an exclusive region of its own segment. HS, horizontal septum; MS, myoseptum. Scale bar, 20 μm . *a*, After the CaP growth cone reached the ventral edge of its segment, it turned laterally and rostrally, and grew dorsally along the lateral surface of the muscle, in the rostral myoseptum, to the level of the horizontal septum. Varicosities formed along the lateral branch at regular intervals, giving it the appearance of a string of beads. *b*, The axon of the MiP motoneurone extended along the medial surface of the dorsal muscle, in the middle of the segment. When the axon reached the dorsal edge, it turned laterally and rostrally, and grew along the lateral surface of the muscle in the rostral myoseptum, to the level of the horizontal septum, elaborating a string of varicosities along the lateral portion of the axon. *c*, The arbor of the RoP motoneurone extended from the medial surface to the lateral surface along the horizontal septum.

animals in which several primary motoneurones of the same type were labelled in more than one segment (Fig. 4), axons in caudal segments preceded axons in rostral segments in half of the cases (38 segments, 7 animals). Second, observations of initial outgrowth of CaP axons using Nomarski optics showed that any trunk segment could be the first to have a CaP growth cone¹⁴ and the appearance of growth cones in other segments did not follow any particular sequence. Thus, rostro-caudal timing seems to be an unlikely explanation of segmental specificity, as axonal outgrowth of a primary motoneurone in any particular segment may precede or follow axonal outgrowth of the primary motoneurone of the same type in adjacent segments.

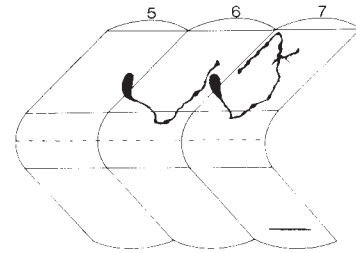


Fig. 4 Axon outgrowth does not occur in a strict rostral-caudal sequence. Two MiP motoneurones were labelled in adjacent segments in the same animal. The growth cone of the caudal MiP (segment 7) grew out of the spinal cord at 19 h PF. The rostral MiP (segment 6) first sprouted a growth cone 1.5 h later. This drawing of the two cells made at 25 h PF shows that the axon of the caudal MiP had already grown into the myoseptum while the rostral axon was still traversing the dorsal muscle. In other animals the opposite order of outgrowth was seen. Scale bar, 25 μm .

After leaving the spinal cord, all three types of motor growth cones initially followed the same pathway to the horizontal septum, where they paused and then selected neurone-specific pathways. Although pausing at particular locations is behaviour typical of growth cones, the wide range of durations involved suggested that pausing itself may not be a crucial component of proper pathfinding. The choice of divergent pathways at the horizontal septum by these growth cones is reminiscent of the choice of neurone-specific pathways by the growth cones of G and C cells in embryonic grasshoppers¹⁵. This type of behaviour is consistent with a 'labelled pathways' hypothesis¹⁶ in which growth cones recognize specific molecular factors, along the axonal pathway, that provide guidance cues to them as they navigate towards their targets. Our results suggest that such labelled pathways may be present in vertebrates.

Some of this work has appeared elsewhere in abstract form¹⁷. This study was inspired by the pioneering work of Speidel¹⁸. We thank David Brumbley, Harrison Howard, James McMurray and Rachel Waga for technical assistance, and Corey Goodman, Charles Kimmel, Lynn Landmesser and Walter Metcalfe for comments on earlier versions of the manuscript. This work was supported by NIH grants NS21132 and NS17963, the Oregon MRF, and an MDA postdoctoral fellowship to J.S.E.

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A T3-like protein complex associated with the antigen receptor on murine T cells

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Antigen recognition by human T lymphocytes and initiation of T-cell activation are mediated by a group of integral membrane proteins, the T-cell antigen receptor (TCR) and the T3 complex¹⁻⁸. The polypeptides which comprise T3 (a γ -chain of relative molecular mass (M_r) 25,000 (25K), and δ and ϵ chains of 20K each) are physically associated with the TCR chains⁹⁻¹². Surface expression of the complex requires the presence of all the component T3 and TCR proteins¹³. In contrast to the human system, murine T3 has not been identified using antibodies. Here we describe a murine T3-like protein complex. It appears to be more complicated than human T3, containing three monomeric glycoproteins (21–28K), two of which have *N*-linked carbohydrate side chains and a novel family of TCR-associated homo- and heterodimers. The 28K protein is identified as the murine T3 δ -chain. The 21K protein is phosphorylated on cell activation with concanavalin A (Con A).

Given the significant structural and functional association of T3 with the human TCR, we expected that T3-like proteins

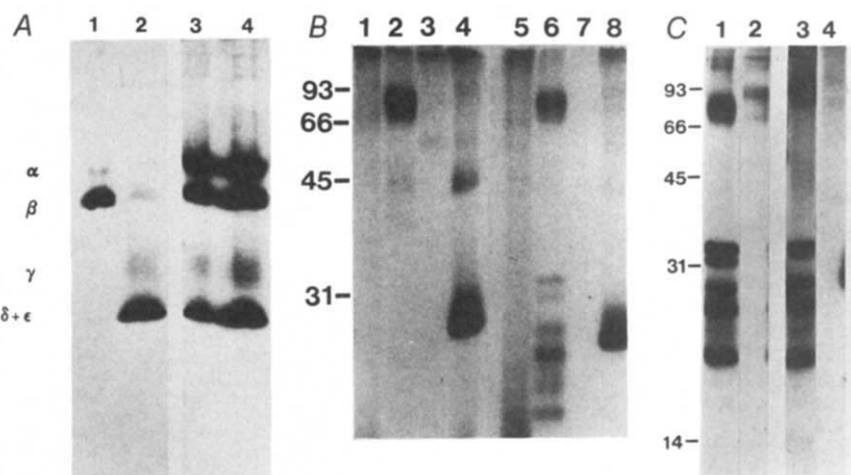
would be present on murine T cells. Recently, a murine complementary DNA clone homologous with the cDNA sequence encoding one of the human T3 chains (T3 δ) has been described¹⁴. We attempted to identify murine T3-like proteins by isolating polypeptides associated with TCR.

For this purpose, it was necessary to improve the methods for extraction of the protein complex, as immunoprecipitation from conventionally prepared lysates of labelled human T cells with anti-TCR (α and β) reagents results in only a minimal co-isolation of the T3 γ , δ and ϵ chains (see Fig. 1A, lane 1). Figure 1A (lanes 3 and 4) shows that when a digitonin-based extraction procedure is used, both anti-T3 and an anti-TCR reagent precipitate the complete series of T3 and TCR polypeptides— α (49K), β (40K), γ (25K) and δ/ϵ (20K)—from ¹²⁵I-radiolabelled cells of the human T-cell leukaemia line HPB-ALL.

Using this method, proteins associated with the murine TCR were isolated from ¹²⁵I-labelled 2B4 cells, a pigeon cytochrome-c-specific, I-E^k-restricted T-T hybridoma¹⁵. As shown in Fig. 1B (lane 2), TCR prepared from Nonidet P-40 (NP40)-solubilized cells appears as a single band of 85–90K. The TCR immunoprecipitate from the digitonin lysate (Fig. 1B, lane 6), however, contains an additional set of six specific bands with apparent M_r s of 34K, 31K, 27K, 25K, 22K and 20K. None of these bands has the same mobility as Thy-1 (Fig. 1B, lane 8) nor do any of them co-precipitate with Thy-1. This control was included because G7, the anti-Thy-1 reagent used for precipitation, causes T-cell proliferation, which suggests that Thy-1 represents the murine homologue of T3 (ref. 16). Studies with another murine T-cell hybridoma, DO-11.10, which is specific for ovalbumin peptides and is I-A^d-restricted², gave the same result using two different TCR-specific antibodies (Fig. 1C). The proteins are not recovered if antibody against major

Fig. 1 A, Human T3 and TCR analysed by SDS-PAGE. ¹²⁵I-surface-labelled HPB-ALL cells were solubilized in isotonic buffer containing 1% NP40 (lanes 1, 2) or 1% digitonin (lanes 3, 4). Immunoprecipitates were prepared with the anti-TCR reagent T40/25 (ref. 23) (lanes 1, 3) and the T3-specific antibody anti-Leu 4 (ref. 24) (lanes 2, 4) and analysed by SDS-PAGE on a 12.5% gel under reducing conditions. B, TCR-associated proteins in the murine antigen-specific T-cell hybrid 2B4. ¹²⁵I-labelled 2B4 (ref. 15) cells were solubilized in NP40 (lanes 1–4) or digitonin (lanes 5–8) extraction buffer. Immunoprecipitation was performed with non-immune mouse serum (lanes 1, 5), normal rat serum (lanes 3, 7), A2B4-2 (anti-TCR)¹⁵ (lanes 2, 6), and G7 (anti-Thy-1)¹⁶ (lanes 4, 8), and samples were analysed under non-reducing conditions on a 12.5% gel. The positions of standard protein markers are indicated on the left ($M_r \times 10^{-3}$). C, TCR-associated proteins on the T-cell hybrid DO-11.10. Digitonin extracts of ¹²⁵I-labelled DO-11.10 cells were immunoprecipitated with the clone-specific anti-TCR antibody KJ1-26.1 (ref. 2) (lane 1), non-immune mouse serum (lane 2), the anti-TCR-allotype reagent KJ16-133 (ref. 25) (lane 3) and non-immune rat serum (lane 4). A2B4-2 (lane 5) and normal mouse serum (lane 6) immunoprecipitates from digitonin-extracted 2B4 cells are shown for comparison.

Methods. HPB-ALL cells²⁶ and the murine T-cell hybrids 2B4 and DO-11.10 were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). Cells (50×10^6) were labelled with ¹²⁵I (2 mCi, NEN) at room temperature in a 200- μ l volume of phosphate-buffered saline (PBS) containing 4 IU lactoperoxidase (Sigma); 10 μ l of 0.05% H₂O₂ was added at 5-min intervals over a reaction period of 20 min. Cells were washed once in PBS and suspended at 0°C for 15 min in 300 μ l extraction buffer (10 mM triethanolamine, 0.15 M NaCl, pH 7.8 with the protease inhibitors: 10 mM iodoacetamide, 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride and 1 μ g ml⁻¹ each of leupeptin, pepstatin, antipain and chymostatin) containing 1% NP40 or 1% digitonin. Digitonin (Aldrich) was prepared as a 2% stock solution. The solid detergent was added to boiling water, which was stirred for 2 min, cooled, allowed to stand at room temperature for 1 week and then filtered²⁷. The cell extract was centrifuged at 13,000g for 15 min and the supernatant at 100,000g for 30 min before pre-clearing for 16 h with 10 μ l of a 10% suspension of *Staphylococcus aureus*. Immunoprecipitation was performed by incubating the extract for 2–4 h with a 10- μ l packed volume of protein A-Sepharose beads (Pharmacia) precoated with antibody (200 μ l of hybridoma culture supernatant or 5 μ l of ascites). Beads coated with rat antibodies were precoated with rabbit anti-rat immunoglobulin. Immunoprecipitates were washed five times in the same buffer used for cell extraction and subjected to SDS-PAGE, using the method of Laemmli²⁸.



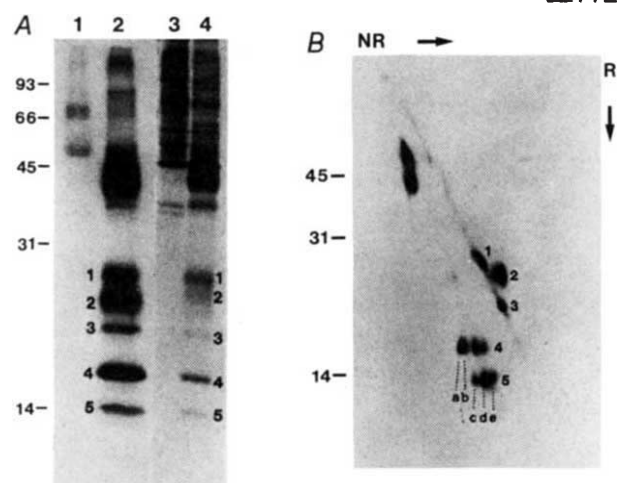


Fig. 2 **A**, Analysis of murine TCR-associated proteins under reducing conditions. DO-11.10 cells were labelled with ^{125}I . 2B4 cells (2×10^6) were incubated at 37°C for 16 h with 1 mCi each of ^{35}S -cysteine and ^{35}S -methionine (Amersham) in 100 ml cysteine/methionine-free RPMI 1640 medium with 10% dialysed FCS. Immunoprecipitates were prepared from digitonin extracts of the DO-11.10 (lanes 1, 2) and 2B4 (lanes 3, 4) cells using normal rat serum (lane 1), normal mouse serum (lane 3) and the anti-TCR reagents KJ16-133 (lane 2) and A2B4-2 (lane 4), and analysed by SDS-PAGE under reducing conditions. **B**, Two-dimensional non-reducing/reducing SDS-PAGE analysis of murine TCR-associated proteins. An A2B4-2 immunoprecipitate from digitonin-extracted ^{125}I -labelled 2B4 cells was applied to a 12.5% SDS-polyacrylamide tube gel and electrophoresed under non-reducing conditions. The gel was then equilibrated for 15 min at room temperature in reducing sample buffer containing 5% 2-mercaptoethanol before application to a 12.5% slab gel.

histocompatibility complex (MHC) class I antigen (K^k) or an inappropriate anti-TCR reagent is used (data not shown). We conclude that the murine TCR is associated with a complex group of proteins, some of which may be murine homologues of the human T3 chains.

When analysed under reducing conditions, five TCR-associated proteins—28K (1), 25K (2), 21K (3), 17K (4) and 14K (5)—were found (Fig. 2A, lane 2). This result was also obtained after biosynthetic labelling with ^{35}S -cysteine and ^{35}S -methionine (Fig. 2A, lane 4). The 31K and 34K proteins present under non-reducing conditions (Fig. 1B, C) were absent and two new small polypeptides of 14K and 17K (4 and 5) were observed. To determine the relationship between the proteins seen in non-reducing conditions (Fig. 1B) and those recovered after reduction (Fig. 2A, 1–5), we performed a two-dimensional non-reducing/reducing SDS-polyacrylamide gel electrophoresis (PAGE) analysis (Fig. 2B). Proteins composed of disulphide-linked subunits appear below the diagonal on such a gel. A series of 17K and 14K spots corresponding to bands 4 and 5 seen under reducing conditions was observed below the diagonal; these spots were aligned such that they appeared to be derived from two 17K homodimers (which we designate *a* and *b*), two heterodimers (14K+17K; *c* and *d*) and a 14K homodimer (*e*). The 21K, 25K and 28K monomeric proteins, seen as bands 1, 2 and 3 on reducing SDS-PAGE analysis of the TCR-associated proteins, were observed as three spots lying on or near the diagonal. The position of protein 2 above the diagonal may be the result of reduction of internal disulphide bonds. We conclude that the murine T3-like complex consists of five proteins, three monomers and two small subunits which form a family of homo- and heterodimers.

The presence of carbohydrate in the T3-like proteins was assayed by labelling with ^3H -mannose. In addition to the TCR α - and β -chains, three bands corresponding to the three TCR-

associated monomers (1, 2 and 3) contained the carbohydrate label (Fig. 3A, lane 4). The dimers containing the 14K and 17K subunits (4 and 5) were not labelled. To confirm the presence of oligosaccharide side chains, the enzyme endoglycosidase F (Endo-F), which hydrolyses the second glycosidic bond of *N*-linked oligosaccharides, was used to digest the purified TCR-associated proteins. Treatment of both the 21K and 28K bands (bands 1 and 3) gave rise to 16K products (Fig. 3B, lanes 2, 6). The bulk of the 25K protein (2) was not altered by Endo-F (Fig. 3B, lane 4). As expected, the 17K subunit (Fig. 3B, lane 7) was unaffected by the deglycosylating enzyme (lane 8). Sufficient amounts of pure 14K protein (band 5) for analysis could not be obtained but in digestions of the unseparated mixture of TCR-associated proteins its mobility had been observed to be unaltered (data not shown). We conclude that proteins 1 and 3, seen as 28K and 21K bands under reducing conditions, contain *N*-linked carbohydrate. Although the 25K protein was not digested, we hesitate to rule out the presence of *N*-linked glycans; incorporation by the 25K protein of ^3H -mannose, a sugar rarely present in *O*-linked carbohydrate, suggests that it may contain *N*-linked glycosylation that is resistant to enzyme digestion. The 14K and 17K subunits appear to be unglycosylated.

The identity of the T3 δ -chain among the murine TCR-associated proteins was determined by immunoblotting using a rabbit antiserum specific for a 17-residue synthetic peptide corresponding to the amino-acid sequence deduced for a murine T3 δ cDNA clone¹⁴. The serum bound specifically to a 28K membrane protein (Fig. 3C, lanes 3, 4). No binding was observed in the presence of competing peptide (lane 5). Pretreatment of the membrane proteins with Endo-F resulted in a decrease in size of the band from 28K to 16K (Fig. 3C, lanes 6, 7). A preparation of ^{125}I -labelled TCR-associated proteins was run in parallel (lane 2). In the microslab system used in this analysis, the 25K and 28K proteins (bands 1 and 2) were not resolved but were seen as a single band of the same size as the specifically immunoblotted protein. Given that the protein detected by antiserum to a T3 δ peptide and the 28K protein (band 1), but not the 25K protein (band 2), are Endo-F-sensitive and have 16K protein backbones, we conclude that the 28K TCR-associated protein represents murine T3 δ ; this is in agreement with the presence of three sites for *N*-linked glycosylation indicated by the murine T3 δ cDNA sequence.

In view of the putative role of T3 in signal transduction and the involvement of protein kinases in cellular proliferation, we examined the possibility that one of the T3-like chains is phosphorylated. A TCR-associated phosphoprotein has been described previously by Samelson and colleagues¹⁷. We were interested to determine whether this polypeptide corresponded to one of the ^{125}I -labelled proteins of the T3-like complex that we had observed. ^{32}P -loaded 2B4 cells either treated with Con A or left unstimulated were solubilized in digitonin and anti-TCR immunoprecipitates were prepared (Fig. 4). Although the background bands were of similar intensity in treated and untreated cells, a 20K ^{32}P -labelled protein which aligned with the 20K ^{125}I -labelled band (band 3) was seen in the anti-TCR immunoprecipitate from the lysate of stimulated cells alone (Fig. 4, lane 4). Whether this observation indicates that band 3 is a protein kinase is being investigated.

We have identified a T3-like complex of proteins associated with TCR in murine T-cell hybrids. The three glycosylated monomers, the smallest of which is phosphorylated on activation, are similar in size and in their TCR-association to the human T3 γ , δ and ϵ chains and may constitute their murine homologues. The largest of these monomers appears to be the murine T3 δ chain. Two of the proteins reported here may correspond to the 24K and 29K proteins isolated with TCR from a murine lymphoma line treated with a crosslinking reagent before solubilization^{18–20}. A 23K protein occasionally observed to co-precipitate with TCR from 2B4 cells, which appears to

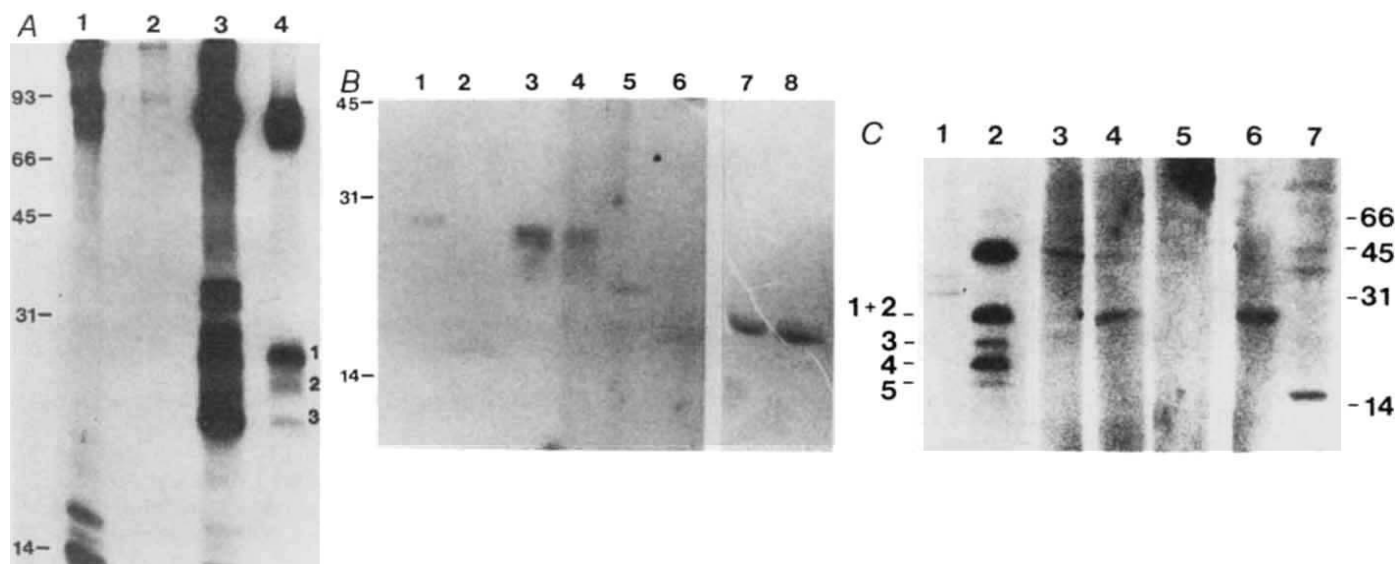


Fig. 3 A, Glycosylation of the murine TCR-associated proteins. 2B4 cells surface-labelled with ^{125}I as described in Fig. 1 (lanes 1, 3) or biosynthetically labelled with ^3H -mannose by incubating 5×10^7 cells for 16 h at 37°C in 10 ml of glucose-poor RPMI (containing 100 mg l^{-1} glucose and 10% dialysed FCS) with 1 mCi ^3H -mannose (Amersham) (lanes 2, 4) were extracted in 1% digitonin solubilization buffer. Normal mouse serum (lanes 1, 2) and A2B4-2 anti-TCR (lanes 3, 4) immunoprecipitates were analysed under non-reducing conditions on a 12.5% gel. The sugar was not metabolically converted to amino acids during the overnight incubation because murine H-2 heavy chain but not β_2 -microglobulin (nonglycosylated) was labelled by ^3H -mannose (data not shown). B, Endo-F digestion of individual murine TCR-associated proteins. 17K, 21K, 25K and 28K proteins were recovered from lane 2 of the gel in Fig. 2A by electroelution²⁹, lyophilized and suspended in 60 μl of 100 mM Tris-HCl pH 6.8, 0.5% SDS, 10 mM EDTA and 1% 2-mercaptoethanol. After heating to 100°C for 3 min, NP40 was added to 1% and protease inhibitors (see Fig. 1 legend) were added. Endo- β -N-acetylglucosaminidase-F (Endo-F)³⁰ (from Dr J. Kaufman) was added to half of each sample and digestion was allowed to proceed for 4 h at 37°C . 6 μl of 10% SDS, 50% glycerol, 40% 2-mercaptoethanol and 0.1% bromophenol blue were added to the treated and untreated samples before application to a 12.5% gel. Lane 1, 28K minus Endo-F; lane 2, 28K + Endo-F; lane 3, 25K - Endo-F; lane 4, 25K + Endo-F; lane 5, 21K - Endo-F; lane 6, 21K + Endo-F; lane 7, 17K - Endo-F; lane 8, 17K + Endo-F. C, Immunoblot analysis of murine T-cell membrane proteins with an anti-T3 δ serum. Immunoprecipitates prepared from digitonin-extracted ^{125}I -labelled DO-11.10 cells using normal mouse serum (lane 1) and the anti-TCR reagent KJ16-133 (lane 2) were separated by SDS-PAGE under reducing conditions on a 12.5% microslab gel³¹. Plasma membrane proteins from the murine thymoma line, EL-4, were run in lanes 3-6. Lane 7 contains Endo-F-digested EL-4 membrane proteins. The proteins were transferred to nitrocellulose³². The strips corresponding to the lanes containing T-cell membrane proteins were blocked in 1.5% gelatin, then incubated with preimmune rabbit serum (lane 3) or rabbit antiserum directed against a synthetic peptide based on the deduced amino-acid sequence (residues 126-142) of the murine T3 δ cDNA (lanes 4-7). Excess free peptide was included in one incubation (lane 5). Nitrocellulose strips were developed with ^{125}I -goat F (ab')₂ anti-rabbit immunoglobulin (NEN).

cause an increase in the size of TCR after crosslinking of surface proteins, has been reported by Samelson and Schwartz²¹. On the basis of its TCR association and M_r , the 23K protein probably corresponds to one of the monomeric T3-like polypeptides of 2B4 cells described here. In addition to the monomers, which appear to be similar to the human T3 chains, we have described a novel family of TCR-associated homo- and heterodimers. It is of interest to note that although both the 14K and 17K subunits are easily surface-labelled with ^{125}I using lactoperoxidase or biosynthetically labelled with ^{35}S -amino acids, only the 17K polypeptide incorporates the hydrophobic-partitioning, photoactivatable label 3-[trifluoromethyl]-3-[*m*-iodophenyl]-deazirine²² (TID), suggesting that it contains a hydrophobic domain that is absent from the 14K protein (data not shown). The possibility that the 14K protein is derived from the 17K protein by *in vivo* or *in vitro* proteolysis remains to be investigated. We believe that a comparison of the structure, biosynthesis and regulation of gene expression of these TCR-associated polypeptide chains in both species will help to elucidate the molecular events involved in TCR function.

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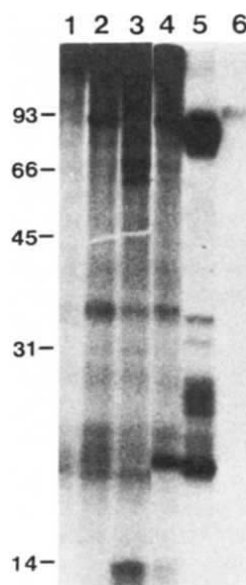


Fig. 4 Phosphorylation of the 21K murine TCR-associated protein. Cells were loaded with ^{32}P and activated as described by Samelson¹⁷. 2B4 cells (1.5×10^8) were washed three times in phosphate-free RPMI 1640 medium and incubated in 8 ml of the same medium containing 10% dialysed FCS and 5 mCi of ^{32}P -orthophosphoric acid for 2 h at 37°C . Con A was then added to $10 \mu\text{g ml}^{-1}$ to one half of the cells for 1 h, after which the untreated and treated cells were washed twice in ice-cold PBS containing the phosphatase inhibitors 0.4 mM sodium vanadate (ortho), 0.4 mM EDTA, 10 mM sodium fluoride and 10 mM sodium pyrophosphate and then extracted with 1% digitonin buffer containing phosphatase inhibitors. Immunoprecipitates were prepared with normal mouse serum (lanes 1, 3) and A2B4-2 (lanes 2, 4) from lysates of the unstimulated (lanes 1, 2) and stimulated (lanes 3, 4) cells. A2B4-2 and normal mouse serum immunoprecipitates from an ^{125}I -labelled extract were run in parallel (lanes 5, 6).

GM07753-06. C.L.P. is a fellow and C.T. a scholar of the Leukemia Society of America.

Note added in proof: Since submission of this letter, Samelson and colleagues have reported similar findings concerning T3-like polypeptides on murine T cells³³, although they differ from us in their interpretation of the protein representing the murine T3 δ chain.

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Retrovirus-mediated transfer and expression of drug resistance genes in human haematopoietic progenitor cells

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Patients with certain genetic disorders can be cured by bone marrow transplantation^{1,2}. However, as prospective donors do not exist for most patients with potentially curable genetic abnormalities, an alternative treatment for such patients involves the transfer of cloned genes into the patient's haematopoietic stem cells followed by re-infusion of the treated cells³. Retroviral vectors provide an efficient means for transferring genes into mammalian cells and have been used to transfer genes into mouse haematopoietic cells⁴⁻⁸. We have now produced amphotropic retroviral vectors containing either the bacterial gene for neomycin resistance or a mutant dihydrofolate reductase gene that confers resistance to methotrexate and have used these vectors to infect and confer drug resistance to human haematopoietic progenitor cells *in vitro*. Transfer could be demonstrated in the absence of helper virus by using an amphotropic retrovirus packaging cell line, PA12 (ref. 9). These studies are an important step towards the eventual application of retrovirus-mediated gene transfer to human gene therapy and for molecular approaches to the study of human haematopoiesis.

The haematopoietic system consists of a hierarchy of cells with differing capacities for self-renewal, proliferation and

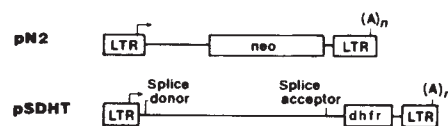


Fig. 1 Retrovirus vectors. The *dhfr*-containing virus pSDHT (ref. 16) contains the coding region for a MTX-resistant *dhfr* gene¹⁵ inserted into a clone of a spleen focus-forming virus in place of the gp52 envelope gene. The messenger RNA encoding *dhfr* is presumably a spliced message, as is the envelope mRNA. The *neo*-containing virus pN2 (ref. 7) contains a neomycin resistance gene¹⁴ inserted between the long terminal repeats (LTRs) and viral replication signals from Moloney murine leukaemia virus. Arrows indicate promoters, (A)_n indicates a polyadenylation signal. High-titre amphotropic retrovirus stocks were generated from these constructs as described previously¹⁶. Briefly, Psi-2 ecotropic retrovirus packaging cells²² were transfected with viral DNA, and virus was collected after 2 days and used to infect PA12 amphotropic retrovirus packaging cells. Drug-resistant clones were isolated and screened for virus production. Helper virus was assayed using the S⁺L⁻ assay⁹. The *dhfr*-virus stock was also tested for the presence of helper virus by infecting NIH 3T3 cells with 1 ml of the virus, passaging the cells for 1 month and assaying these cells for production of *dhfr*-virus and helper virus. In two separate experiments, no virus was detected. Vector titre (CFU ml⁻¹) and helper virus titre (focus-forming units per ml) were, respectively, 5 × 10⁶ and >10³ (helper virus titre increased with passage of this cell line) for pN2 and 2 × 10⁶ and <1 for pSDHT.

differentiation. Haematopoietic stem cells are primitive cells that undergo both self-renewal and commitment to form progenitor cells¹⁰. Through extensive proliferation and differentiation of these progenitor cells, a large number of diverse blood cells is formed. Progenitor cells that can be grown *in vitro* include those which give rise to granulocyte/monocyte colonies (CFU-GM)¹¹, erythroid colonies (BFU-E)¹² and mixed colonies containing both erythroid and myeloid elements (CFU-MIX)¹³. Certain haematological disorders, such as adenosine deaminase deficiency, purine nucleoside phosphorylase deficiency and the haemoglobinopathies, are potentially curable by transferring normally functioning genes into pluripotent haematopoietic stem cells of patients with such disorders. An important step towards this type of therapy is the demonstration that genes can be efficiently transduced into human haematopoietic cells. Although gene transfer into pluripotent haematopoietic stem cells cannot be measured *in vitro*, we expect that successful transfer into haematopoietic progenitor cells will be indicative of our ability to infect stem cells.

We used retroviral vectors (Fig. 1) containing either a neomycin resistance gene (*neo*)¹⁴ or a mutant dihydrofolate reductase gene (*dhfr*)¹⁵, dominant-acting selectable genes that confer resistance to the antibiotic G418 or the antifolate methotrexate (MTX), respectively, in mammalian cells. High-titre amphotropic retrovirus stocks were generated from these vectors by transfer into PA12 cells as described previously¹⁶. Several PA12-SDHT cell lines produced *dhfr*-containing virus at 2 × 10⁶ colony-forming units (CFU) ml⁻¹ in the absence of helper virus. Several PA12-N2 cell lines produced *neo*-containing virus in excess of 2 × 10⁶ CFU ml⁻¹, but these lines also produced amphotropic helper virus. Helper virus production by these cells was unexpected because the PA12 packaging cells do not produce helper virus when other vectors are used (refs 9, 16, and unpublished results). Helper virus production was apparently due to a recombination event between homologous overlapping sequences in the *neo*-virus and the virus packaging system which are not present in most vectors¹⁶.

We next determined the concentrations of MTX and G418 which would completely inhibit the growth of human haematopoietic colonies *in vitro*. In agreement with previous reports¹⁷, little inhibition of CFU-GM colony growth was seen with MTX concentrations of 10⁻⁴ M when either dialysed or undialysed fetal bovine serum was included in the incubation

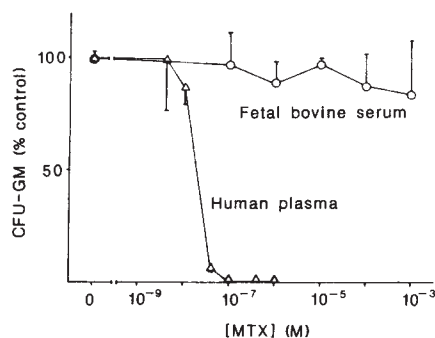


Fig. 2 Effect of methotrexate on CFU-GM colony growth. Human bone marrow samples were obtained from adult volunteers who were donating their marrow for allogeneic bone marrow transplantation. The bone marrow was fractionated by density gradient centrifugation on Ficoll-Hypaque²³. The mononuclear cell fraction was removed and washed with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Bone marrow cells (1×10^5) were incubated in 35-mm dishes containing 1 ml of Iscove's modified Dulbecco's medium with $40 \mu\text{l ml}^{-1}$ placental conditioned medium²⁴, 0.3% agar, either 20% heat-inactivated (1 h, 56°C) FBS ($\circ$) or 20% heat-inactivated human AB plasma (Δ) and MTX as indicated. After incubation for 10–14 days, colonies containing >40 cells were counted using an inverted phase microscope. The total number of colonies from duplicate (FBS-containing plate) or quadruplicate wells (human AB plasma-containing plates) were counted and the results are expressed as a percentage of control plates not receiving MTX ($\pm$ s.d.). Values are representative of several experiments.

mixture. However, when human plasma was used as the source of serum growth factors, the growth of colonies containing both granulocytic and monocytic elements was stimulated and complete inhibition of colony growth was seen at 10^{-7} M MTX (Fig. 2), which correlates with the clinical observations showing bone marrow suppression with MTX serum levels of 10^{-7} M (ref. 18). Methotrexate did not consistently inhibit BFU-E and CFU-MIX growth, however, probably because of the complex nature of the medium required for their growth. In contrast, complete growth inhibition of all colony types was demonstrable with G418 at a concentration of 2 mg ml^{-1} for CFU-GM and 1 mg ml^{-1} for BFU-E and CFU-MIX.

Efficient gene transfer into mouse haematopoietic cells has been achieved only when the bone marrow cells have been co-cultivated with virus-producing cells. Co-cultivation of human bone marrow cells with *neo*- or *dhfr*-virus-producing cells resulted in conversion of 3–10% of the CFU-GM to drug resistance (Table 1). No drug-resistant colonies were seen when bone marrow cells were co-cultivated with parental PA12 cells, which produce viral particles lacking viral RNA. Resistant colonies contained mixtures of myeloid elements including granulocytes and monocytes/macrophages, as confirmed by Wright-Giemsa staining of individual colonies after their removal from agar. Co-cultivation of bone marrow cells with monolayers of *neo*-virus-producing cells conferred G418 resistance to $\sim 20\%$ of the BFU-E. In addition, co-cultivation of marrow cells with the *neo*-virus-producing cells conferred G418 resistance to CFU-MIX, demonstrating that this marrow progenitor cell can also be infected. However, the scarcity of this marrow progenitor cell prevented us from obtaining an accurate estimate of the frequency of infection. As with the CFU-GM, co-cultivation with parental PA12 cells did not confer drug resistance to BFU-E or CFU-MIX.

The presence of helper virus in the *neo*-virus preparation used here (Fig. 1) allowed us to confirm the presence of the *neo*-virus in G418-resistant haematopoietic colonies by rescue of *neo*-virus from these cells. For assay of *neo*-virus release, individual CFU-GM colonies were removed from agar, dispersed by pipetting and co-cultivated with NIH 3T3 (thymidine kinase-negative) cells. After overnight incubation, the culture medium was

Table 1 Efficiency of gene transfer into CFU-GM, BFU-E and CFU-MIX using retroviral vectors

Progenitor cell Selective agent	CFU-GM G418	
	PA12	PA12/N2
Co-cultivation with:	0/605 (0%)	27/624 (4.3%)
	0/544 (0%)	31/583 (5.3%)
	0/455 (0%)	15/417 (3.6%)
Totals	0/1,320 (0%)	73/1,624 (4.5%)
Progenitor cell Selective agent	CFU-GM MTX	
	PA12	PA12/SDHT
Co-cultivation with:	0/246 (0%)	26/256 (10%)
	0/320 (0%)	11/331 (3.3%)
	0/331 (0%)	13/327 (4.0%)
	0/240 (0%)	19/254 (7.5%)
Totals	0/1,137 (0%)	69/1,168 (5.9%)
Progenitor cell Selective agent	BFU-E G418	
	PA12	PA12/N2
Co-cultivation with:	0/365 (0%)	33/282 (12%)
	0/302 (0%)	48/305 (16%)
	0/1,086 (0%)	313/1,409 (22%)
	0/1,435 (0%)	264/1,336 (20%)
Totals	0/3,188 (0%)	658/3,332 (20%)
Progenitor cell Selective agent	CFU-MIX G418	
	PA12	PA12/N2
Co-cultivation with:	0/7 (0%)	1/9 (11%)
	0/3 (0%)	1/4 (25%)
	0/— (0%)	2/4 (50%)
Totals	0/10 (0%)	4/17 (24%)

Control fibroblasts (PA12) and virus-producing fibroblasts (PA12/N2 and PA12/SDHT) were trypsinized and seeded at 5×10^5 per 6-cm dish the day before use. Bone marrow cells, enriched for mononuclear cells as described in Fig. 2 legend, were co-cultivated with fibroblasts for 16 h at 37°C in 4 ml of fresh medium (DMEM supplemented with 10% FBS). Fibroblasts were irradiated (caesium source, $0.459 \text{ Gy min}^{-1}$ for 5 min) before addition of the bone marrow cells, to ensure that fibroblast colonies would not grow in soft agar culture and be confused with haematopoietic colonies. After co-cultivation, the non-adherent cells were removed and collected by centrifugation (400g, 5 min). The assays described in Fig. 2 legend were used to detect G418- and MTX-resistant CFU-GM, BFU-E and CFU-MIX colonies were grown in Iscove's modified Dulbecco's medium containing 30% FBS (Hyclone, heat-inactivated), 1% beef embryo extract (Gibco), 1% bovine albumin (Armour), 5×10^{-5} M 2-mercaptoethanol, 100 U ml^{-1} penicillin, $100 \mu\text{g ml}^{-1}$ streptomycin, 0.1 mg ml^{-1} NaHCO_3 , $5 \mu\text{l ml}^{-1}$ placental conditioned medium, 0.9% methylcellulose and 1 IU ml^{-1} human urinary erythropoietin, essentially as described previously^{12,13}. CFU-E were assayed in the absence or presence of either 2 mg ml^{-1} G418 or 10^{-7} M MTX. BFU-E and CFU-MIX were assayed in the absence and presence of 1 mg ml^{-1} G418. After incubation for 14 days, colonies were counted using an inverted microscope. The number of colonies per 10^5 mononuclear cells was 25–100 for CFU-GM, 50–190 for BFU-E and 0.5–2 for CFU-MIX. Each set of paired values is from a separate experiment and represents the number of colonies from quadruplicate wells. The number of drug-resistant colonies is listed first, followed by the number of colonies which grew in the absence of drug.

replaced with medium containing 2 mg ml^{-1} G418 and after 7 days the plates were scored for G418-resistant colonies. Of 12 G418-resistant colonies previously exposed to *neo*-virus, 3 induced G418-resistant NIH 3T3 colonies, demonstrating the presence of the *neo*-virus in these colonies. We were not surprised that only a few colonies produced *neo*-virus, for it means that these colonies have been infected with both helper virus and *neo*-virus, which may be a rare event. Thus, the transfer of the *neo* gene into human bone marrow progenitor cells was demonstrated by two criteria, the conferral of drug resistance to CFU-GM, BFU-E and CFU-MIX and the recovery of *neo*-virus from the individual CFU-GM colonies.

These results show that retroviral vectors can be used to transfer genes into normal human haematopoietic progenitor cells. Co-cultivation of bone marrow cells with virus-producing cells results in drug-resistant human CFU-GM with an efficiency (3–10%) similar to those observed for mouse (10–20%)⁷ and dog (10–25%)¹⁹ marrow. As the same vector (N2) was used to infect progenitor cells from all three species, we conclude that the efficiency of infection of human marrow is comparable to that of other animals. Because we have been able to infect all specimens examined so far (from over 15 individuals) using these vectors, we also conclude that marrow from most individuals should be susceptible to gene transfer by amphotropic retroviral vectors.

For studies of human haematopoiesis using retroviruses and for eventual application of retroviruses to human gene therapy, it is important to transfer genes in the absence of helper virus, because this prevents replication and spread of the vector following transfer and eliminates the possibility of helper virus-induced disease. The *dhfr*-virus used here was free of replication-competent helper virus by two stringent criteria (Fig. 1) and conferred resistance on CFU-GM with a similar efficiency to the helper virus-containing *neo*-virus. We conclude that replication-defective vectors can successfully mediate gene transfer in human haematopoietic cells in the absence of helper virus. In addition, we assayed all progenitor cells for drug resistance immediately after short-term exposure to virus, therefore drug resistance is due to the initial infection of these cells and not to viral spread, even in the case of the helper virus-containing *neo*-virus. Gene transfer into human CFU-GM has previously been demonstrated only after long-term culture of haematopoietic cells with virus vectors containing helper virus^{20,21}.

The finding that the *dhfr*-virus confers MTX resistance on haematopoietic progenitor cells at levels of MTX which affect haematopoiesis *in vivo* suggests that selection for the presence of the vector *in vivo* will be possible. Cells infected with viruses containing the *dhfr* gene linked to a non-selectable gene may similarly be enriched *in vivo*. Hence, even though infection may not be 100% efficient, selection pressure *in vivo* may greatly increase the number of cells containing and expressing the transduced genes.

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Structural alteration of viral homologue of receptor proto-oncogene *fms* at carboxyl terminus

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A role for proto-oncogenes in the regulation and modulation of cell proliferation^{1,2} has been suggested by the findings that the B-chain of platelet-derived growth factor (PDGF) is encoded by the proto-oncogene *sis*^{3–7} and that the *erb-B* oncogene product is a truncated form of the epidermal growth factor (EGF) receptor^{8–10}. Furthermore, the product of the proto-oncogene *fms* (*c-fms*) may be related or identical to the receptor for macrophage colony-stimulating factor (CSF-1)¹¹. *v-fms* is the transforming gene of the McDonough strain of feline sarcoma virus (SM-FeSV)^{12,13} and belongs to the family of *src*-related oncogenes which have tyrosine-specific kinase activity. Furthermore, nucleotide sequence analysis of the *v-fms* gene product revealed topological properties of a cell-surface receptor protein¹⁴. To elucidate the features involved in the conversion of a normal cell-surface receptor gene into an oncogenic one, we have now determined the complete nucleotide sequence of a human *c-fms* complementary DNA. The 972-amino-acid *c-fms* protein has an extracellular domain, a membrane-spanning region, and a cytoplasmic tyrosine protein kinase domain. Comparison of the feline *v-fms* and human *c-fms* sequences reveals that the proteins share extensive homology but have different carboxyl termini.

A λ gt10 cDNA library of $\sim 2 \times 10^6$ clones prepared from total human term placental poly(A)⁺ RNA was screened at moderate stringency using a radioactively labelled *Clal*/*Bgl*II (2.66 kilobases; kb) fragment of *v-fms* (pSM-FeSV) as a hybridization probe (see Fig. 1 legend). Seventeen clones were isolated which were derived from the same messenger RNA, as indicated by restriction analysis and sequence homology to *v-fms*. Figure 1 shows the complete nucleotide sequence of overlapping cDNA clones and translation of the longest open reading frame initiating with methionine. The 3,992-nucleotide sequence is divided into 300 bases of 5'-untranslated sequence, 2,916 nucleotides of coding sequence, and a 716-nucleotide 3'-untranslated region containing a poly(A) addition signal (ATTAAA) 16 base pairs (bp) upstream from the poly(A) tail of several independent clones. Two other cDNA clones (*lc-fms* 32 and *lc-fms* 5) have extended 3'-untranslated regions and contain another poly(A) addition signal 47 bp upstream of the poly(A) tail (not shown). The human placenta thus contains at least two species of *c-fms* mRNAs which have different 3' ends. The longest cDNA sequence is 4,291 nucleotides, which is close to the size determined for the mRNA on Northern blots (4.3 kb), and may well contain all of the *c-fms* mRNA sequence.

The 972-amino-acid primary translational product of *c-fms* sequence contains features characteristic of cell-surface glycoprotein receptors. These include an amino-terminal, predominantly hydrophobic stretch of 23 amino acids, starting with the putative initiator methionine at nucleotide positions 301–303. The sequence surrounding the methionine codon matches the criteria for a likely initiation codon (GCCATGG; ref. 15). An in-frame termination codon (TGA) is found 252 bp upstream from this putative initiation codon, with no alternative ATG in between. Met 1 is thus likely to be the *c-fms* initiation methion-

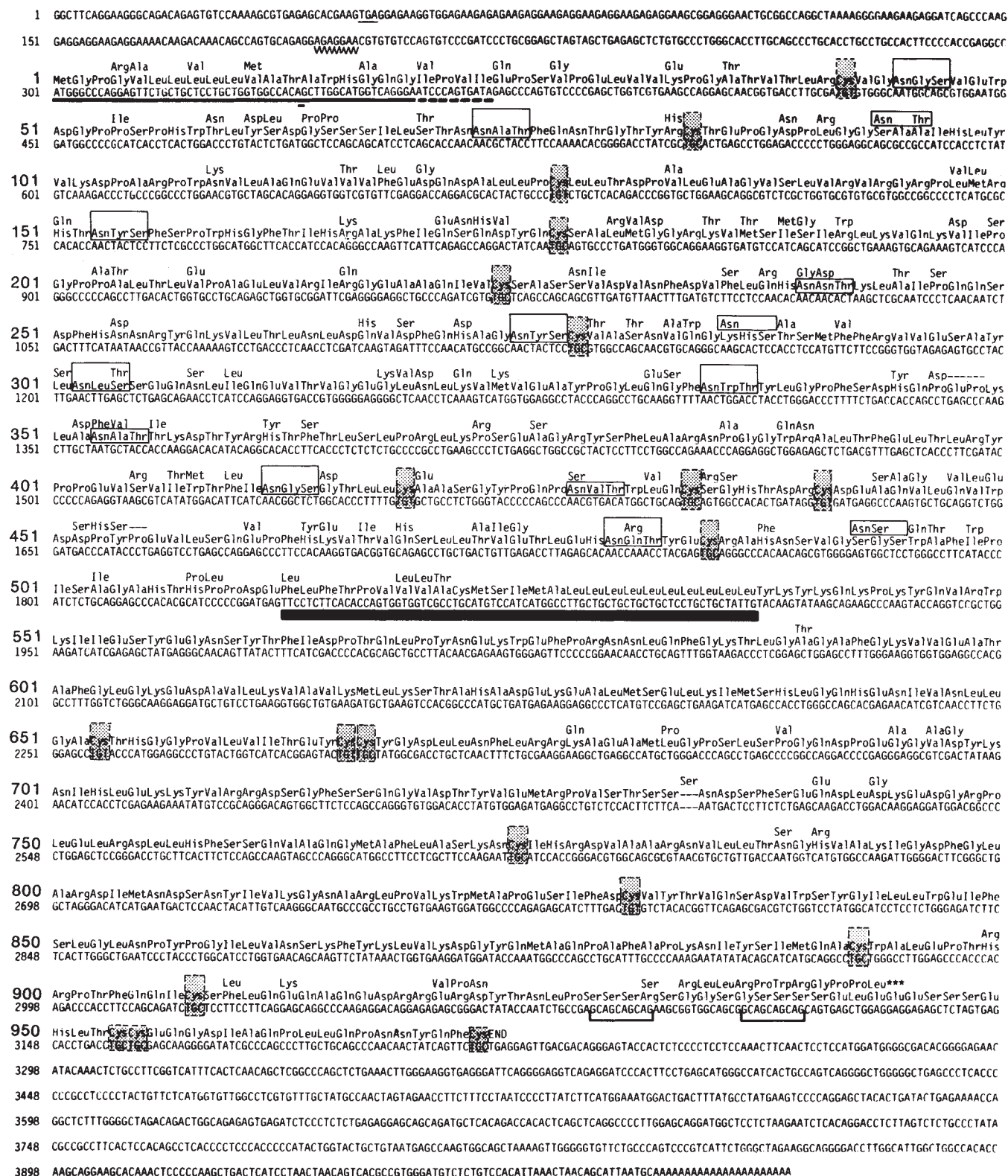


Fig. 1 Nucleotide sequence of *c-fms* cDNA. The nucleotide and amino-acid numbers are shown on the left. In the coding domain, the amino-acid numbers are shown above the nucleotide number. The 5' in-frame termination codon, TGA, is indicated by thin underlining; the sequence at the recombination junction between *FeLV* and the *v-fms* gene is underlined with a wavy line; the putative signal sequence is indicated with a thicker line followed by a broken line; cysteine residues are shaded; putative N-linked glycosylation sites are shown by open boxes; the transmembrane region is emphasized by a black bar; two direct repeats are bracketed; the poly(A) addition signal is underlined and marked by an arrow.

Methods. Total poly(A)-containing RNA was isolated from frozen human term placenta. Using the λ gt10 vector system²⁶, a cDNA library containing 1.5×10^6 recombinants was constructed⁹. Initial screening of this library used a radiolabelled²⁷, 2.15-kb *Clal/BglII* fragment isolated from the *v-fms* clone pSM-FeSV/*Clal*/BamHI (2.66 kb)¹². Two plaque pure positive phage were characterized by Southern blot hybridization and sequenced^{28,29}. A 1.5-kb *EcoRI* fragment from phage λ -fms 32 was then used to rescreen the library and detected 151 putative positive phage. This pool was then screened with a 5'-proximal 130-bp fragment (*Clal/HincII*) from *v-fms* pSM-FeSV/*Clal*/BamHI. The longest of the 27 phage isolated, λ -fms 102, was sequenced and found to contain the entire pre-*c-fms* coding sequence preceded by an open reading frame. λ -fms 305 was found to extend further 5' and to contain an in-frame stop codon.

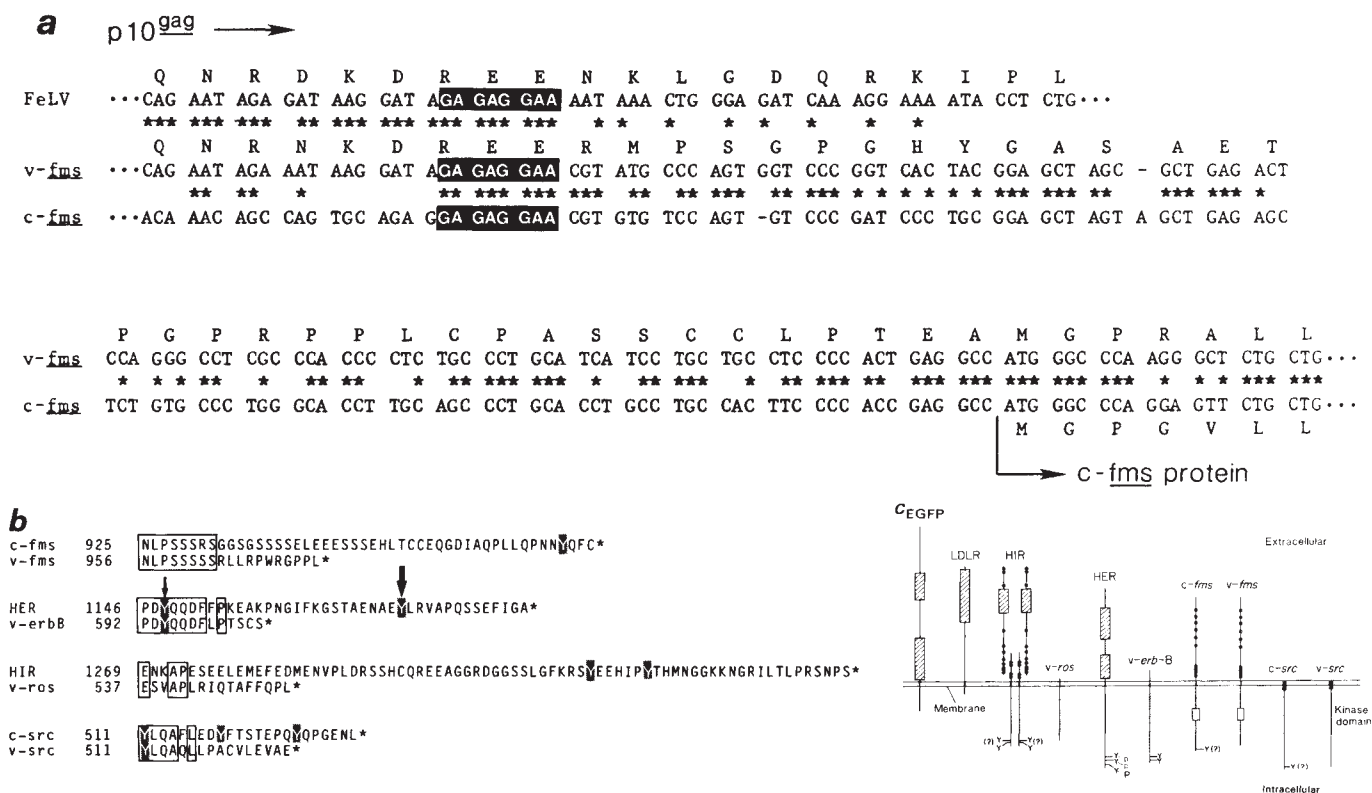


Fig. 2 **a**, *v-fms* and *c-fms* recombination junction. The sequence around the putative recombination junction of FeLV³⁰ and *c-fms* (Fig. 1) used to generate *v-fms* is shown. Eight common nucleotides are boxed. The reading frame in FeLV and *v-fms* is translated in single-letter code. Nucleotide sequence homologies are indicated by asterisks. The putative initiation codon of the *c-fms* coding sequence is indicated. **b**, Carboxy-terminal sequence differences between receptors and their oncogenic derivatives. The carboxy-terminal sequences of human *c-fms* and feline *v-fms*, human epidermal growth factor receptor (HER) and avian *v-erb-B*⁸, and avian *c-src* and *v-src*²⁴ are shown. Open boxes indicate homology; tyrosine residues are indicated by solid boxes. Arrows on tyrosine residues indicate phosphorylation sites; the solid filled arrow denotes the major autophosphorylation site of human EGF receptor²³. **c**, Schematic comparison of structural features of cell-surface receptors and oncogene products. Regions of high-Cys concentration are shown as hatched boxes and single Cys residues as filled circles. The open box in *v-fms* and *c-fms* represents a 70-amino-acid substitution in the tyrosine-kinase domain. The position of carboxy-terminal tyrosine residues is shown. EGFP, epidermal growth factor precursor; LDLR, low-density lipoprotein receptor; HIR, human insulin receptor; HER, human epidermal growth factor receptor.

ine, followed by an ~19–23-amino-acid signal sequence, required for transfer of the nascent polypeptide chain into the lumen of the endoplasmic reticulum¹⁶. The predicted relative molecular mass (M_r) of the unglycosylated *c-fms* polypeptide is 105,000 and 106,000 for the 949- or 953-residue protein, respectively.

During its transport to the cell surface, the feline *c-fms* protein is glycosylated to yield a 165,000- M_r glycoprotein¹⁷. Eleven N-linked glycosylation target sites are found within the 512-residue amino-terminal half of the human *c-fms* sequence (Fig. 1), which represents the extracellular ligand binding domain of this glycoprotein receptor. The *c-fms* extracellular domain lacks the cysteine-rich repeat units¹⁸ found in the cell-surface receptors for EGF⁸, insulin¹⁹ and low-density lipoprotein (LDL)²⁰. Although the cysteine content is much lower in *c-fms*, six amino-proximal residues are regularly spaced and are flanked by related sequences, which suggests the possibility of a symmetrically folded conformation within the extracellular ligand binding domain. Hydrophobicity analysis of the *c-fms* sequence reveals a fivefold, 90–100-amino-acid repeat of hydrophobicity markers, which may indicate the presence of five similarly folded subdomains in this part of the *c-fms* polypeptide (not shown).

A stretch of 25 amino acids characteristic of a membrane-spanning anchor domain separates extracellular and cytoplasmic parts of the sequence. At its carboxyl terminus, the transmembrane sequence is followed by a highly polar and basic

stretch of residues (Tyr-Lys-Tyr-Lys-Gln-Lys-Pro-Lys-Tyr), which may function as a 'stop transfer' sequence and possibly interact with negatively charged phospholipid headgroups on the cytoplasmic surface of the membrane. No threonine or serine residues are found in this region, which could correspond to threonine 654 of the EGF receptor, thought to be involved in protein kinase C modulation of that receptor's activity⁶. The closest potential target for protein kinase C interaction is located at Ser 555, eight residues downstream from the target in the human EGF receptor, Thr 654.

The cytoplasmic domain of the *c-fms* protein is about 530 amino acids long, and shares extensive homology with members of the family of tyrosine-protein kinases⁷. These homologies include the probable ATP binding domain at positions 587–596 and lysine at 616. The homology in the kinase domain is interrupted by a 70-amino-acid insertion in *c-fms* at residue 670, and fades around residue 910 towards the carboxyl terminus. Interestingly, the spacing between the transmembrane and the protein kinase domains is identical to that observed in EGF receptor and insulin receptor^{8,19}. The *c-fms* protein from macrophages has been shown to be phosphorylated at tyrosine residues on addition of its presumptive ligand, CSF-1 (ref. 21).

Comparison of the human *c-fms* cDNA and derived amino-acid sequences with corresponding feline *v-fms* sequences reveals a high degree of sequence conservation, especially when one considers the evolutionary distance between these species. The 972-amino-acid *c-fms* sequence is 84% homologous with

v-fms. The most significant differences are found at both the amino-terminal and carboxy-terminal ends. Extracellular sequences display the lowest level of homology (75%) and cytoplasmic domain sequences the highest homology (95%), until *c-fms* and *v-fms* diverge completely, shortly before the carboxyl terminus at residue 933 (see Fig. 1). The sequence differences in the extracellular domain include two in-frame deletions in *v-fms* of one and two codons, respectively (positions 348, 349 and 455). It remains possible that the sequence differences in this domain, of which ~60% are non-conservative, result in altered ligand affinity or specificity.

The open reading frame encodes the 972-amino-acid *c-fms* protein and extends 249 nucleotides beyond the putative initiation codon into the 5'-untranslated sequence. This region includes a ~150-residue purine-rich sequence that starts about 100 nucleotides upstream from the beginning of the coding sequence and contains short sequence repeats such as GAAGA or GGAAG (Fig. 1). Sequence comparisons revealed that an 8-nucleotide sequence (GAGAGGAA) at the point of divergence between *c-fms* and *gag-v-fms* was also found in the relevant position of the *gag* gene sequence of feline leukaemia virus (FeLV-B)³⁰ (Figs 1, 2a). This sequence may mark the point where a homologous recombination event generated the *gag-v-fms* gene. This event truncated the *gag* gene, and fused it in-frame with the pre-*c-fms* sequence. 5'-Untranslated sequences of *c-fms* were thus converted into *gag-v-fms* coding sequences.

Although Hampe *et al.*¹⁴ have previously proposed that a leader sequence required to generate a glycosylated *gag*-encoded protein acts as a signal sequence for the *gag-fms*-encoded protein, our data suggest the alternative, that the signal sequence of *c-fms* acts as an internal signal for membrane translocation in the *gag-v-fms* fusion polypeptide. This also offers a possibility for the as yet unknown processing mechanism of gp180^{gag-fms} to generate p55^{gag} and gp120^{fms} by signal peptidase cleavage at the end of the *c-fms* leader sequence.

We have localized the 3' junction between *c-fms* and FeLV to the *pol* gene at a point 595 nucleotides upstream from the *env* gene initiation codon (results not shown), by comparison with sequences of FeLV-B (J. Elder, personal communication). This is consistent with previous observations that the *env* gene product of SM-FeSV is intact^{12,13}. No homologous or overlapping sequences were discerned when *v-fms* or *c-fms* sequences were compared with ~500 nucleotides constituting the 3' end of the feline *pol* gene and 5' end of the *env* gene. Inspection of *v-fms* and *c-fms* sequences reveals that the two diverge completely at amino acid 933. The *c-fms* protein extends for 40 additional amino acids, while the *v-fms* coding sequence terminates after 11 unrelated codons of unknown origin (Figs 1, 2b).

The region in which the carboxyl termini of *v-fms* and *c-fms* sequences diverge is flanked by two GCAGCAGCAG repeats (Fig. 1) which may have been involved in sequence truncation by recombination. When the sequence of another *fms*-containing retrovirus, HZ5-FeSV, was compared with the *v-fms* and *c-fms* sequences described here, the point of sequence divergence between the two *v-fms* sequences was identical to that shown in Fig. 1. Homology between *c-fms* and the HZ5-FeSV *v-fms* gene continues beyond the point of divergence between *c-fms* and the SM-FeSV *v-fms* genes (P. Besmer, personal communication). The carboxyl terminus of the *v-fms* protein of HZ5-FeSV is also altered, but differs from that of SM-FeSV, thus excluding the possibility that the carboxyl-termini differences are due to species-specific divergence. As two *c-fms*-derived oncogenes have undergone structural alterations at their carboxyl termini, this may represent an important event in the conversion of the cellular *fms*/CSF-1 receptor gene into a gene capable of causing transformation *in vitro* and tumours *in vivo*.

Figure 2b, c compares the tyrosine-kinase family oncogenes, *v-erb-B*, *v-fms* and *v-src* and their cellular relatives, the EGF receptor, *c-fms*/CSF-1 receptor and *c-src*. All three viral

oncogenes have an altered carboxyl terminus compared with their putative cellular cognate. The *v-ros* gene product²², which is closely related to the insulin receptor, also has an altered carboxyl terminus compared with the *c-ros* gene product (L.-H. Wang, personal communication). In all cases the carboxyl termini of cellular cognates contain tyrosine residues, which have an important role in signal transduction. A tyrosine residue is found at position 969 of *c-fms* in a location similar to that for the EGF receptor tyrosine 1,173, which is a major autophosphorylation substrate for the ligand-induced tyrosine-autophosphorylation activity²³. Interestingly, the corresponding tyrosine is also missing from the deduced *v-fms* protein encoded by either SM-FeSV or HZ5-FeSV. This is also true of Tyr 527 in the *c-src* protein, which is missing from *v-src* because of an altered carboxyl terminus²⁴.

Oncogenes of the transmembrane tyrosine kinase type (*v-ros*, *v-erb-B*, *v-fms*) differ in their extracellular domains (Fig. 2c). *v-ros* and *v-erb-B* are related to polypeptide hormone receptors that possess cysteine-rich sequence repeats in their extracellular ligand-binding domains^{8,19}. This family includes the non-tyrosine-kinase LDL receptor²⁰, as well as the EGF precursor, which may be a receptor for an unknown ligand in addition to being the precursor for EGF^{18,25}. Both *v-ros* and *v-erb-B* lack most of these extracellular ligand-binding domain sequences, including the cysteine-rich repeats. In contrast, *c-fms* retains the entire putative ligand-binding domain of *c-fms*/CSF-1 receptor. Interestingly, this glycoprotein is not a member of the receptor family containing the cysteine-rich clusters. This difference between *v-fms* and *v-ros*/*v-erb-B* may reflect alternative mechanisms used by the respective receptor molecules to control transmembrane signal generation. Double truncation of cellular *ros* and *erb-B* proto-oncogenes may be necessary to generate a transforming gene, while a single carboxyl-terminal truncation of the *c-fms* gene may yield the same result.

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Genomic diversity correlates with clinical variation in Ph'-negative chronic myeloid leukaemia

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The Philadelphia chromosome (Ph') is found in the blood cells of about 90% of patients with chronic myeloid leukaemia (CML) and usually results from the reciprocal chromosome translocation $t(9; 22)^{1,2}$. This translocation relocates the proto-oncogene *c-abl*, normally found on chromosome 9q34, to within the breakpoint cluster region (*bcr*) on chromosome 22q11 (refs 3–8). The juxtaposition of *c-abl* and the 5' portion of *bcr* appears to be the critical genomic event in CML and results in a novel 8-kilobase (kb) fused *abl/bcr* transcript^{9,10} and a *c-abl*-related protein of relative molecular mass 210,000 (ref. 11). About 10% of adult patients diagnosed as CML lack the Ph' chromosome; they represent a heterogeneous group of disorders which are difficult to diagnose precisely¹². We have examined five patients with CML whose leukaemic cells have a normal karyotype. We report here that two of the patients showed the same genomic change as occurs in Ph'-positive CML, but the change resulted from a mechanism other than chromosomal translocation. The remaining three patients showed no genomic rearrangement. This genomic diversity correlated with the clinical differences between the patients.

The *abl* oncogene is located on chromosome 9 and previous *in situ* hybridization studies have shown that it is translocated to the Ph' chromosome in CML^{3–6}. *In situ* hybridization of an ³H-labelled *v-abl* probe¹³ to metaphase cells of a Ph'-negative patient (patient 1) resulted in specific labelling of the terminal

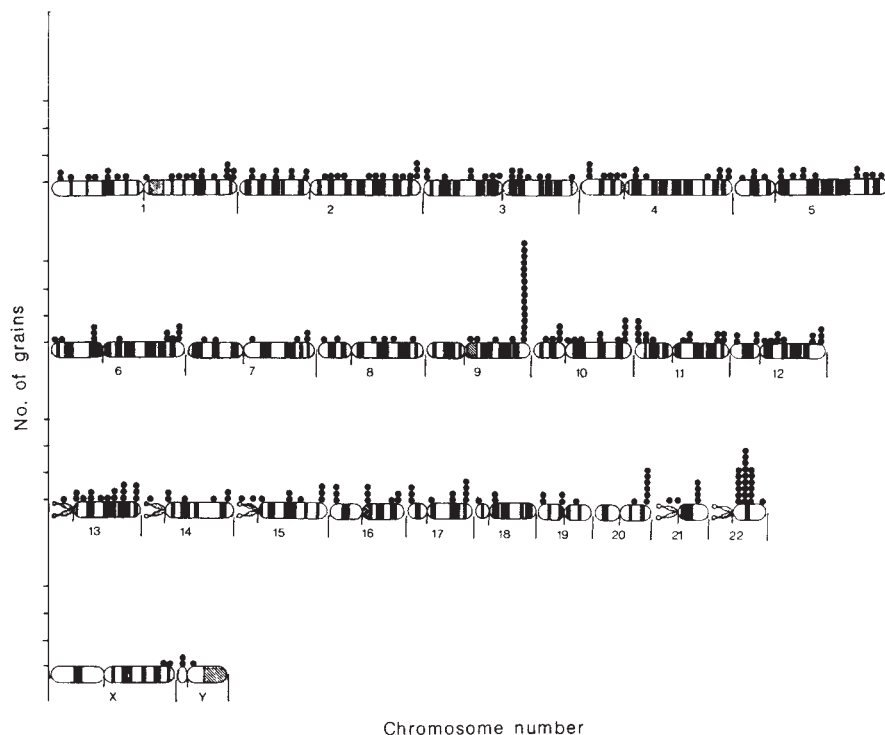
region of chromosome 9q and the proximal half of chromosome 22q. Figure 1 shows the distribution of labelled sites in 138 metaphase cells, representing the combined result of two *in situ* hybridizations to cultured leukaemic blood cells. All G-banded and R-banded cells had a normal karyotype. Sixteen cells were labelled at band 9q34 of chromosome 9, and 21 cells were labelled in the proximal half of chromosome 22q. The probability of each of these distributions was <0.1% on the basis of unit chromosome length and also on the basis of the number of grains per labelled chromosomal site. This is the same oncogene shift as occurs in the standard Ph' translocation, but the chromosomes 22 of our patient showed no morphological evidence of translocation and this was confirmed by *in situ* studies which showed that *c-sis* remained on chromosome 22 (Fig. 2). In Ph'-positive CML, *c-sis* is translocated from chromosome 22 to chromosome 9 (ref. 14). Furthermore, a *bcr* probe which has homology with the 3' end of *bcr* was also present on chromosome 22 and not translocated to chromosome 9q (Fig. 2) as in the standard $t(9; 22)^{7,8}$. These results suggest an insertion of DNA carrying *c-abl* in an otherwise normal chromosome 22.

Restriction enzyme analysis of DNA from patient 1, using the 3' *bcr* (*Hind*III–*Bgl*II) plasmid probe⁷, showed the presence of a new fragment in *Bam*HI, *Bgl*II and *Bgl*II–*Xho*I digests of leukaemic DNA compared with control DNA from the same patient (Fig. 3, lanes 1–2, 5–6 and 9–10, respectively). *Hind*III and *Bgl*II–*Sac*I digests were identical in leukaemic and control DNA (Fig. 3, lanes 3–4, 7–8). These results suggest the presence of a break in the *bcr* gene ~0.6 kb downstream of exon 3 and between the *Xho*I and *Sst*I restriction sites (Fig. 5); such a break separates the 5' and 3' portions of *bcr* and would allow, in our patient, insertion of *c-abl* adjacent to the 5' portion of *bcr*, a position that appears to be characteristic of CML and essential for production of the 8-kb hybrid *abl-bcr* RNA transcript^{9,10}.

Restriction enzyme analysis of leukaemic DNA from patient 2 using the same *bcr* probe showed rearrangements in *Hind*III, *Bam*HI and *Eco*RI digests (Fig. 4, lanes 1, 2, 4), but not in a *Bgl*II digest (Fig. 4, lane 3). A *Bgl*II–*Sac*I digest also demonstrated a *bcr* rearrangement (data not shown). We conclude that in patient 2 a breakpoint occurred within the *bcr* gene between the *Hind*III and *Bgl*II sites (Fig. 5). Both patients 1 and 2 showed the same type of rearrangements as occur in the 5.8-kb *bcr* region following the relocation of *c-abl* to the *bcr* region in

Fig. 1 Distribution of sites labelled with the *v-abl* probe in leukaemic cells from patient 1. Major clusters of grains occur on chromosome 9 at 9q34 and on chromosome 22 at 22q11–12. A small cluster of grains was also observed at band 20q12 ($P < 1\%$), suggesting that cross-hybridization to *c-src* had occurred; the *c-src* probe shows a similar cross-hybridization to *c-abl* (ref. 24).

Methods. Leukaemic cells were cultured and mitoses synchronized by the method of Yunis²⁵ and G-banded²⁶. All cells had a normal karyotype. The psub-9 *v-abl* probe¹³ was labelled by nick-translation to a specific activity of $1.6\text{--}3 \times 10^7$ d.p.m. μg^{-1} using ³H-dATP and ³H-dCTP. *In situ* hybridization procedures were essentially as described elsewhere²⁷. The metaphase cells scored had an average number of two grains (upper range 5) over the chromosomes, and approximately the same numbers on the background.



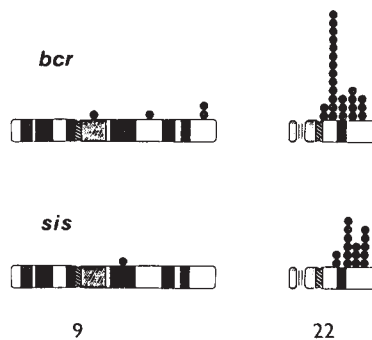


Fig. 2 Distribution of sites labelled with the 3'-*bcr*⁷ and *v-sis* probes²⁸ on chromosomes 9 and 22 of leukaemic cells from patient 1. ³H-labelling of probes and *in situ* hybridization were done as described in Fig. 1 legend.

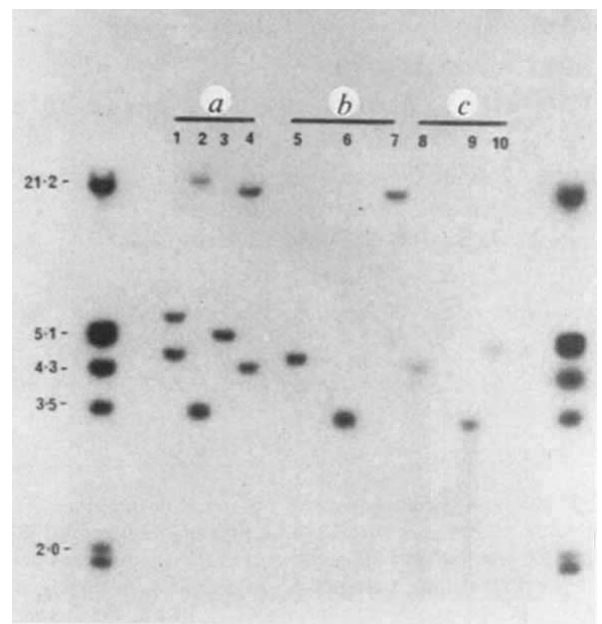


Fig. 4 Southern blot hybridization of a *bcr* probe to DNA from leukaemic cells of patients 2-4 (*a-c*, respectively). DNA samples were digested with *Hind*III, *Bam*HI or *Eco*RI (*a*, lanes 1-4 respectively); *Hind*III, *Bam*HI and *Eco*RI (*b*, lanes 5-7); and *Hind*III, *Bam*HI and *Bgl*II (*c*, lanes 8-10). Southern blots were prepared and processed as described in Fig. 3 legend.

Ph⁺-positive CML^{7,8}, yet the leukaemic cells of both patients 1 and 2 were Ph⁻-negative and had normal karyotypes.

Three further Ph⁻-negative patients were studied and none of them showed the gene rearrangements characteristic of Ph⁺-positive CML. Hybridizations using the *bcr* probe showed no rearrangements in *Hind*III, *Bam*HI or *Eco*RI digests of leukaemic DNA from patient 3 (Fig. 4, lanes 5-7) or in *Hind*III, *Bam*HI and *Bgl*II digests from patient 4 (Fig. 4, lanes 8-10). *In situ* hybridization of the ³H-labelled *v-abl* probe to chromosomes of leukaemic cells from patient 5 showed specific labelling only at the terminal region of chromosome 9q—the same result as that obtained for a normal control (data not shown).

Two of our Ph⁻-negative CML patients showed the same genomic changes as occur in Ph⁺-positive CML; the other three patients did not. This genomic heterogeneity correlated with the clinical variation shown by patients presently diagnosed as being

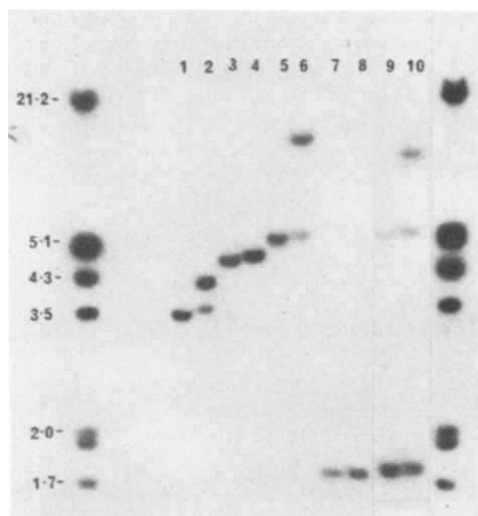
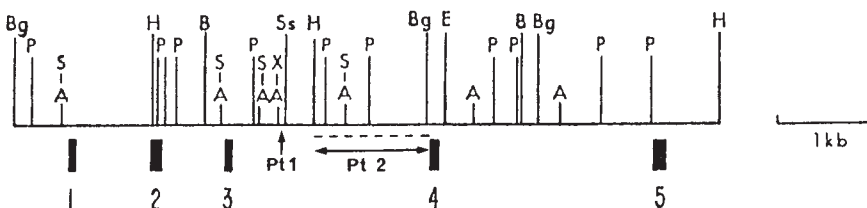


Fig. 3 Southern blot hybridization of the *bcr* probe to DNA from leukaemic cells of patient 1. Control DNA from Epstein-Barr virus-transformed lymphocytes of patient 1 is shown in the odd-numbered lanes and leukaemic DNA in even-numbered lanes. The DNA samples were digested with the following enzymes: *Bam*HI (lanes 1, 2), *Hind*III (lanes 3, 4), *Bgl*II (lanes 5, 6), *Bgl*II-*Sac*I (lanes 7, 8), and *Bgl*II-*Xho*I (lanes 9, 10). Southern blots were prepared²⁹ and hybridized with a nick-translated³⁰ 1.2-kb *Hind*III-*Bgl*II *bcr* insert⁷. Filters were washed at 68 °C in 0.1×SSC, 0.5% SDS. λ markers (*Hind*III-*Eco*RI) are indicated on the left (in kb). Further studies indicated that the 5.1-kb bands in lanes 9 and 10 were the result of modification of the *Xho*I site rather than partial digestion.

Ph⁻-negative¹². These patients usually present at an older age, have monocytosis, thrombocytopenia, a poor response to chemotherapy and a shortened survival, but a subgroup of Ph⁻-negative CML patients show clinical and haematological features which are indistinguishable from patients with the Ph⁺ chromosome¹⁵⁻¹⁸. Our patients 1 and 2 were of the latter type, and it is significant that these patients showed essentially the same genomic rearrangement as occurs in Ph⁺-positive cases, while retaining an apparently normal karyotype. The clinical courses of patients 1 and 2 have not differed in any way from that seen in Ph⁺-positive CML. These patients, who are 26 and 49 yr old, are in the chronic phase of CML (6 yr and 4 yr respectively after diagnosis). Both also showed cell colony growth characteristic of CML when buffy coat blood cells were plated on soft agar with colony-stimulating factor (CSF)¹⁹. Our remaining three patients (3-5) represented the more usual type of Ph⁻-negative CML: all three were ≥ 70 yr old at diagnosis and had a monocytosis in the blood of $4-14 \times 10^9 \text{ l}^{-1}$. All responded poorly to treatment with progressive thrombocytopenia and none survived for more than 10 months. Blood cells of patients 3 and 4 did not show the distinctive CML pattern in soft agar with CSF¹⁹ (patient 5 was not tested). These three patients differed clinically and genomically from true CML patients and they probably were suffering from a different haematological disease: a case can be made for their inclusion in the myelodysplastic syndrome^{12,20}.

True CML is characterized by the juxtaposition of *c-abl* and *bcr*, and the use of molecular probes will allow the accurate diagnosis of CML patients, whether or not they have a visible chromosomal translocation. Movement of *c-abl* from chromosome 9q to chromosome 22 has been demonstrated not only in the standard Ph⁺ translocation t(9;22), but also in several variant and complex translocations^{4-6,21}. Some of the cases with complex translocations appear to be Ph⁻-negative, but they carry 'masked' Ph⁺ chromosomes²¹⁻²³. Our results show that *c-abl* and *bcr* can be associated in Ph⁻-negative CML without apparent chromosomal translocation, but as the result of a separate mechanism which relocates DNA within the cell.

Fig. 5 Restriction enzyme map of the breakpoint cluster region (*bcrl*) of chromosome 22 after Heisterkamp *et al.*⁸. The sites of breakpoints found in patients 1 and 2 are indicated. The broken line represents the location of the 1.2-kb *bcrl* probe used in these experiments. Enzymes used to construct the map were: *Ava*I (A), *Bam*HI (B), *Bgl*III (Bg), *Eco*RI (E), *Hind*III (H), *Pst*I (P), *Sma*I (S), *Sst*I or *Sac*I (Ss) and *Xho*I (X)⁸.



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Sequence homology of the yeast regulatory protein ADR1 with *Xenopus* transcription factor TFIIIA

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Classical yeast genetics coupled with the cloning of regulatory genes by complementation of function^{1,2} is a powerful means of identifying and isolating *trans*-acting regulatory elements³⁻⁶. One such regulatory gene is *ADR1* which encodes a protein required for transcriptional activation of the glucose-repressible alcohol dehydrogenase (*ADH2*) gene^{7,8}. We now report the nucleotide sequence of *ADR1*; it encodes a polypeptide chain of 1,323 amino acids, of which the amino-terminal 302 amino acids are sufficient to stimulate *ADH2* transcription. This active amino-terminal region shows amino-acid sequence homology with the repetitive DNA-binding domain of TFIIIA⁹, an RNA polymerase III transcription factor of *Xenopus laevis*¹⁰. Similar domains are found in proteins encoded at the *Krüppel*¹¹ and *Serendipity*¹² loci of *Drosophila melanogaster*. We discuss the implications of this structural homology and suggest that a similar domain may exist in other yeast regulatory proteins such as those encoded by *GAL4* (ref. 13) and *PPR1* (ref. 14).

Figure 1 shows the complete nucleotide sequence of *ADR1* and the predicted amino-acid sequence of the encoded protein. In addition to 3,969 base pairs (bp) of coding-region DNA, 610 bp of 5'-noncoding and 1,560 bp of 3'-noncoding sequence were determined. The presumed initiator methionine codon is flanked by sequences favourable for initiation of translation in eukaryotes^{15,16} and is preceded by two TAA termination codons in the same reading frame. The DNA sequence of *ADR1* encodes a polypeptide of 1,323 amino acids with a calculated relative molecular mass of 150,985.

Table 1 illustrates the codon usage for the *ADR1* gene. In *Saccharomyces cerevisiae* a correlation is observed between high levels of gene expression and a bias towards preferred codons which are complementary to the anticodons of the major isoacceptor transfer RNA species¹⁷. The calculated codon bias index for *ADR1* is very low, 0.076, indicating a lack of preferred

codons and predicting a low level of expression.

Several features of the predicted *ADR1* protein are noteworthy. The amino-acid composition of the amino-terminal region of the protein is highly basic: residues 62-256 are 21% lysine+arginine and only 7% glutamate+aspartate. One portion of this region, residues 203-228, is 41% lysine+arginine. Chou and Fasman conformational parameters¹⁸ predict that residues 214-254 could form an α -helical structure. There are five cysteines between residues 106 and 140, a region that shows striking homology to TFIIIA¹⁹, as described below. Three potential sites of phosphorylation are found in the amino-terminal 230 amino acids of *ADR1*; there are none in the rest of the protein. The sequence Arg-Arg-Ala-Ser at residues 227-230 fits one of the two main patterns of acceptor sites for phosphorylation of the serine residue by bovine type II cyclic AMP-dependent protein kinase²⁰. The sequences Arg-Lys-Asn-Ser at residues 177-180 and Arg-Lys-Ala-Ser at residues 209-212 are also potential phosphorylation sites but have reduced affinity for the type II cyclic AMP-dependent protein kinase²¹.

The most interesting feature of *ADR1* is the apparent structural homology to TFIIIA, a transcription factor for RNA polymerase III from *X. laevis*²². TFIIIA promotes formation of a stable transcription complex by binding to the internal control region of 5S genes¹⁰. The predicted amino-acid sequence of *ADR1* was used in a homology search conducted for us by Dr Russell Doolittle, using an updated version of the NEWAT database²³; the only significant match was to TFIIIA. The region of sequence homology with *ADR1* is in a region of TFIIIA which binds 5S genes²⁴ (Fig. 2). TFIIIA contains a 30-amino-acid motif repeated nine times in tandem in the DNA-binding domain⁹. The most conserved residues of a repeat unit are two cysteines, two histidines and three invariant hydrophobic residues: tyrosine, phenylalanine and leucine. An acidic residue is present between the cysteines, and basic residues, or residues with DNA-binding side chains, are positioned in the 12-residue stretch between the cysteines and histidines. *ADR1* contains two repeat units at positions 98-126 and 127-155 which match the TFIIIA consensus sequence. The conservation of residue type and position suggests that *ADR1* may have a function similar to that of TFIIIA. It is possible that *ADR1* binds to the upstream activation site (UAS) of *ADH2* (refs 8, 25) through this region of the protein, and establishes a functional transcription complex.

ADR1 and TFIIIA have another feature in common. Following the postulated repeated structure in each protein there is a

-600	-590	-580	-570	-560	-550	-540	-530	-520	-510	-500	-490
TCGATAGTACGCCGTTGTGAACATAAAAGTGATATTTTTCGTTGCTTTTTCGTTGCGGTTGTTTCATTGTTTTCATGTTTACTTCGCCACAGGTTTCAAAGAACAC											
-480	-470	-460	-450	-440	-430	-420	-410	-400	-390	-380	-370
GCCTTAAAGTAAAGAAAGGTTTCGCTACAGGTTGTTTATTGTTGCTGTTGTTTATGCTACTACTGTTGTTTATTCGGACTTCGGATCGGAAATTTCTCCCT											
-360	-350	-340	-330	-320	-310	-300	-290	-280	-270	-260	-250
TGAAGACCTTTTGAAGACAACAGTTATATATCATGCTGAATTTCTCAGGCTATTTTCAAATTCCTACCTCTTATTCACACATTTCCTGACTACTATAGAAAAGCCTTATCTT											
-240	-230	-220	-210	-200	-190	-180	-170	-160	-150	-140	-130
TATCTTTTGAAGAAAGGTTGCTACAGCAAAAGTTTATGTTACTGTTTGTATGCTACTCCCTCTTATTCGTTGGAAGTAAAGATTGATTTCGATAAATTAACCAATCATTTTC											
-120	-110	-100	-90	-80	-70	-60	-50	-40	-30	-20	-10
CTACTTCCCGGTTCTCCCTTTATATAAACAACCTCAGAAAAATTTCTGCTACTATTCCTTACTTACTATAAGAAATTTGTTTCCAAAAAATAAATAAATAATCATAC											
0	10	20	30	40	50	60	70	80			
TCTATTACT ATG GCT AAC GTA GAA AAA CCA AAC GAT TGT TCA GGC TTT CCC GTT GTT GAC TTG AAT TCG TGC TTT TCT AAC GGC TTC AAT											
Met Ala Asn Val Glu Lys Pro Asn Asp Cys Ser Gly Phe Pro Val Val Asp Leu Asn Ser Cys Phe Ser Asn Gly Phe Asn											
90	100	110	120	130	140	150	160	170			
AAT GAG AAA CAA GAA ATA GAA ATG GAA ACG GAT GAT TCA CCG ATT TTA TTA ATG TCA TCA TCA GCT TCC AGA GAA AAC TCA AAC ACT TTC											
Asn Glu Lys Glu Glu Ile Glu Met Glu Thr Asp Asp Ser Pro Ile Leu Leu Met Ser Ser Ser Ala Ser Arg Glu Asn Ser Asn Thr Phe											
180	190	200	210	220	230	240	250	260			
TCT GTG ATA CAG AGC ACG CCA GAT GGA AAG ATC ATT ACC ACA AAT AAT AAT ATG AAC TCC AAG ATT AAC AAG CAA CTG GAC AAG TTG CCC											
Ser Val Ile Glu Arg Thr Pro Asp Gly Lys Ile Ile Thr Thr Asn Asn Asn Met Asn Ser Lys Ile Asn Lys Glu Leu Asp Lys Leu Pro											
270	280	290	300	310	320	330	340	350			
GAA AAT TTA AGG CTT AAT GGT AGA ACC CCC AGT GGG AAA CTA AGG TCA TTT GTT TGC GAG GTT TGT ACG AGA GCG TTC GCA AGA CAA GAG											
Glu Asn Leu Arg Leu Asn Gly Arg Thr Pro Ser Gly Lys Leu Arg Ser Phe Val Cys Glu Val Cys Thr Arg Ala Phe Ala Arg Glu Glu											
360	370	380	390	400	410	420	430	440			
CAC TTG AAA AGA CAT TAC AGA TCG CAT ACA AAT GAA AAA CCT TAT CCC TGT GGC CTC TGC AAC AGA TGC TTT ACT AGG AGG GAC TTA CTG											
His Leu Lys Arg His Tyr Arg Ser His Thr Asn Glu Lys Pro Tyr Pro Cys Gly Leu Cys Asn Arg Cys Phe Thr Arg Arg Asp Leu Leu											
450	460	470	480	490	500	510	520	530			
ATC AGG CAT GCT CAA AAA ATC CAT AGT GGT AAT TTA GGG GAA ACG ATT TCC CAT ACC AAG AAA GTG TCG AGA ACT ATA ACT AAA GCT CGG											
Ile Arg His Ala Glu Lys Ile His Ser Gly Asn Leu Gly Glu Thr Ile Ser His Thr Lys Lys Val Ser Arg Thr Ile Thr Lys Ala Arg											
540	550	560	570	580	590	600	610	620			
AAA AAT TCT GCA TCC TCA GTC AAG TTT CAA ACT CCA ACC TAT GGT ACT CCA GAT AAT GGT AAT TTT TTG AAT CGC ACT ACT GGC AAT ACA											
Lys Asn Ser Ala Ser Ser Val Ser Thr											
630	640	650	660	670	680	690	700	710			
AGA AGA AAA GCA AGC CCT GAA GCT AAT GTT AAA CGT AAG TAC TTG AAA AAA CTG ACG CGC AGG GCT TCA TTT AGC GCA CAA TCA GCA TCC											
Arg Arg Lys Ala Ser Pro Glu Ala Asn Val Lys Arg Lys Tyr Leu Lys Lys Leu Thr Arg Arg Ala Ser Phe Ser Ala Glu Ser Ala Ser											
720	730	740	750	760	770	780	790	800			
AGC TAT GCT TTG CCC GAC CAA TCT TCG CTA GAA CAA CAT CCA AAG GAT CGT GTT AAA TTT TCT ACG CCT GAA TTA GTT CCA CTT GAC TTG											
Ser Tyr Ala Leu Pro Asp Glu Ser Ser Leu Glu Glu Glu Thr Pro Lys Asp Arg Val Lys Phe Ser Thr Pro Glu Leu Val Pro Leu Asp Leu											
810	820	830	840	850	860	870	880	890			
AAG AAT CCT GAA CTT GAC TCT TCG TTT GAC CTG AAT ATG AAT CTA GAT TTA AAC CTA AAT CTA GAT TCC AAT TTC AAT ATA GCA TTA AAC											
Lys Asn Pro Glu Leu Asp Ser Ser Phe Asp Leu Asn Met Asn Leu Asp Leu Asn Leu Asn Leu Asp Ser Asn Phe Asn Ile Ala Leu Asn											
900	910	920	930	940	950	960	970	980			
CGT TCT GAT TCT TCT GGA TCA ACA ATC AAT TTG GAT TAT AAA TTG CCC GAA TCA GCA AAT AAC TAC ACA TAT TCT TCC GGC TCA CCA ACC											
Arg Ser Asp Ser Ser Gly Ser Thr Met Asn Leu Asp Tyr Lys Leu Pro Glu Ser Ala Asn Asn Tyr Thr Tyr Ser Ser Gly Ser Pro Thr											
990	1000	1010	1020	1030	1040	1050	1060	1070			
CGC GCA TAT GTC GGC GCT AAC ACG AAT TCT AAG AAC GGT TCA TTT AAT GAC GCA GAC TTA TTG TCG TCG TCG TAC TGG ATA AAA GCC TAT											
Arg Ala Tyr Val Gly Ala Asn Thr Asn Ser Lys Asn Ala Ser Phe Asn Asp Ala Asp Leu Leu Ser Ser Tyr Trp Ile Lys Ala Tyr											
1080	1090	1100	1110	1120	1130	1140	1150	1160			
AAT GAT CAT TTG TTT TCA GTA TCT GAA AGT GAT GAA ACT TCT CCA ATG AAC TCT GAA TTA AAC GAC ACT AAA TTA ATC GTC CCA GAT TTT											
Asn Asp His Leu Phe Val Ser Glu Ser Asp Glu Thr Ser Pro Met Asn Ser Glu Leu Leu Asn Thr Lys Leu Ile Val Pro Asp Phe											
1170	1180	1190	1200	1210	1220	1230	1240	1250			
AAA TCG ACT ATA CAT CAT TTG AAG GAT TCA AGG TCC TCC TCT TGG ACT GTT GCT ATA GAT AAT AAT AGC AAT AAC AAT AAG GTA TCA GAC											
Lys Ser Thr Ile His His Leu Lys Asp Ser Arg Ser Ser Thr Thr Val Ala Ile Asn Asn Asn Ser Asn Asn Asn Lys Val Ser Asp											
1260	1270	1280	1290	1300	1310	1320	1330	1340			
AAC CAA CCT GAT TTC GTC GAT TTT CAA GAA CTG CTG GAT AAT GAT ACT TTA GGT AAT GAT TTG TTA GAG ACC ACT GGC GTT TTA AAA GAA											
Asn Glu Pro Asp Phe Val Asp Phe Glu Glu Leu Leu Asp Asn Asp Thr Leu Gly Asn Asp Leu Leu Glu Thr Thr Ala Val Leu Lys Glu											
1350	1360	1370	1380	1390	1400	1410	1420	1430			
TTT GAA CTT TTA CAT GAT AGC GTA AGT GCT ACC GCC ACG TCA AAT GAG ATT GAC CTT TCC CAT TTG AAC CTA TCA AAC TCT CCA ATT											
Phe Glu Leu Leu His Asp Asp Ser Val Ser Ala Thr Ala Thr Ser Asn Glu Ile Asp Leu Ser His Leu Asn Leu Ser Asn Ser Pro Ile											
1440	1450	1460	1470	1480	1490	1500	1510	1520			
TCT CCT CAT AAG TTA ATT TAT AAG AAT AAA GAG GGG ACC AAT GAC GAT ATG TTG ATT TCT TTC GGA CTC GAT CAT CCT TCC AAT CGC GAA											
Ser Pro His Lys Leu Ile Tyr Lys Asn Lys Glu Gly Thr Asn Asp Asp Met Leu Ile Ser Phe Gly Leu Asp His Pro Ser Asn Arg Glu											
1530	1540	1550	1560	1570	1580	1590	1600	1610			
GAT GAT CTG GAT AAG CTA TGT AAT ATG ACC AGA GAT GTT CAA GCC ATA TTC AGT CAA TAT TTG AAA GGA GAA GAG TCT AAA CGA TCC CTG											
Asp Asp Leu Asp Lys Leu Cys Asn Met Thr Arg Asp Val Glu Ala Ile Phe Ser Glu Tyr Leu Lys Gly Glu Glu Ser Lys Arg Ser Leu											
1620	1630	1640	1650	1660	1670	1680	1690	1700			
GAA GAC TTT TTA TCA ACG TCA AAC AGG AAA GAA AAG CCA GAT ACG GCC AAC TAT ACT TTT TAT GGG TTA GAT TCT TTA ACG TTA TCG AAA											
Glu Asp Phe Leu Ser Thr Asn Arg Lys Glu Lys Pro Asp Ser Gly Asn Tyr Thr Phe Thr Gly Leu Asp Cys Leu Thr Leu Ser Lys											
1710	1720	1730	1740	1750	1760	1770	1780	1790			
ATA TCA AGA GCT CTG CCG GCC TCC ACT GTG AAC AAC AAT CAG CCA TCG CAT TCC ATA GAA TCA AAG CTA TTT AAT GAA CCA ATG AGA AAT											
Ile Ser Arg Ala Leu Pro Ala Ser Thr Val Asn Asn Asn Glu Pro Ser His Ser Ile Glu Ser Lys Leu Phe Asn Glu Pro Met Arg Asn											
1800	1810	1820	1830	1840	1850	1860	1870	1880			
ATG TGC ATT AAA GTG CTT AGA TAC TAT GAA AAG TTC AGT CAT GAT AGT AGT GAG AGT GTC ATG GAC TCT AAT CCA AAC TTG CTG TCC AAA											
Met Cys Ile Lys Val Leu Arg Tyr Thr											
1890	1900	1910	1920	1930	1940	1950	1960	1970			
GAA TCG TTA ATG CCA GCT GTG AGT GAA TTG AAC GAA TAT TTA GAT CTT TTC AAG AAT AAT TTC CTT CCC CAT TTC CTT ATT ATT CAC CCA											
Glu Leu Leu Met Pro Ala Val Ser Glu Leu Asp Lys Leu Asn Glu Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr											
1980	1990	2000	2010	2020	2030	2040	2050	2060			
AGC TTG CTT GAT TTG GAT TTG GAT AGC TTG CAA CGA TAT ACT AAT GAG GAT GGG TAT GAT GAC GCT GAA AAC GCG CAG TTG TTT GAT CGA											
Ser Leu Leu Asp Leu Asp Leu Asp Ser Leu Glu Arg Tyr Thr Asn Glu Asp Gly Tyr Asp Asp Ala Glu Asn Ala Glu Leu Phe Asp Arg											
2070	2080	2090	2100	2110	2120	2130	2140	2150			
TTA AGT CAA GGG ACA GAT AAA GAA TAT GAT TAC GAG CAC TAT CAA ATC TTG TCC ATT TCG AAA ATC GTT TGT TTA CCC TTA TTT ATG GCC											
Leu Ser Glu Gly Thr Asp Lys Glu Tyr Asp Tyr Glu His Lys Thr Glu Ile Leu Ser Ile Ser Lys Ile Val Cys Leu Pro Leu Phe Met Ala											
2160	2170	2180	2190	2200	2210	2220	2230	2240			
ACA TTT GGT TCT TTG CAT AAG TTC GGT TAC AAA TCT CAA ACA ATA TTG TAT GAG ATG AGT AGA AGA ATT CTA CAT TCT TTT TTG GAG											
Thr Phe Gly Ser Leu His Lys Phe Gly Tyr Lys Ser Glu Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr											
2250	2260	2270	2280	2290	2300	2310	2320	2330			
ACT AAA AGA AGG TGT CGC AGT ACA ACA GTA AAT GAC AGT TAT CAG AAC ATT TCG TTG ATG CAA TCC CTA ATA TTG AGC TTC ATG TTC GCT											
Thr Lys Arg Arg Cys Arg Ser Thr Thr Val Asn Asp Ser Tyr Glu Asn Ile Trp Leu Met Glu Ser Leu Ile Leu Ser Phe Met Phe Ala											

Fig. 1 Nucleotide sequence of the *ADR1* gene and the predicted amino-acid sequence of the encoded protein. The amino-acid sequence of the *ADR1* open reading frame is given below the nucleotide sequence starting with the putative initiation codon, which is designated +1, and ending with a TGA termination codon at +3,970. Two TAA termination codons are present 18 and 27 bp upstream of the ATG initiation codon and define the 5' boundary of the open reading frame. Translation termination codons are found in the other two reading frames within 55 nucleotides of 3'-untranslated and 1,560 nucleotides of 5'-untranslated sequence contain no large open reading frames on either strand. Potential phosphorylation sites in the amino-acid sequence are indicated by thin lines. TFIIIA-like repeats are indicated by thick lines.

Methods. DNA for sequence analysis was derived from plasmid YRp7-ADR1-411 (ref. 5). Small overlapping DNA fragments were cloned into the replicative form of M13mp

phages^{3,4}. Host strain JM103 was used for transformation by recombinant DNAs^{3,4}. Sequence analysis by the dideoxy chain terminator method was initiated with the *EcoRI* fragment (+54 to +1,005) known to lie within the *ADR1* functional region⁵ and extended in both directions to include the *ADR1* open reading frame and 5' and 3' flanking sequences shown. Approximately 80% of the contiguous DNA sequence was derived from both strands of DNA. Where only one orientation of DNA provided unambiguous data, the sequence was determined from two or more different clones. Fidelity of the analysis was checked by cleaving the DNA with most of the restriction enzymes having 6-base recognition sites which were predicted to cut the DNA within the coding region. As another check on the sequence, the *ADR1* gene was fused at the *Bgl*III site at codon 642 to the *lacZ* gene. A fusion protein of the predicted size was made (data not shown), demonstrating that the predicted reading frame was used in yeast.

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2348      2358      2368      2378      2388      2398      2408      2418      2420
CTA GTT GCT GAT TAT TTG GAG AAA ATT GAC TCC TCT TTG ATG AAA AGG CAA TTG TCC GGA TTA TGT TCA ACG ATC AGA TCA AAC TGT TTA
Leu Val Ala Asp Tyr Leu Glu Lys Ile Asp Ser Ser Leu Met Lys Arg Gln Leu Ser Ala Leu Cys Ser Thr Ile Arg Ser Asn Cys Leu

2438      2448      2458      2468      2478      2488      2498      2508      2518
CCG ACA ATT TCT GCA AAT TCT GAG AAG AGT ATC AAT AAT AAC AAT GAA CCT TTA ACA TTT GGT TCT CCT CTT CAA TAC ATC ATT TTT GAG
Pro Thr Ile Ser Ala Asn Ser Glu Lys Ser Ile Asn Asn Asn Asn Glu Pro Leu Thr Phe Gly Ser Pro Leu Gln Tyr Ile Ile Phe Glu

2528      2538      2548      2558      2568      2578      2588      2598      2608
TCA AAA ATT AGA TGC ACC TTA ATG GCT TAT GAT TTT TGT CAG TTC TTG AAA TGT TTC TTC CAT ATT AAA TTC GAT TTG TCT ATA AAG GAA
Ser Lys Ile Arg Cys Thr Leu Met Ala Tyr Asp Phe Cys Gln Phe Leu Lys Cys Phe Phe His Ile Lys Phe Asp Leu Ser Ile Lys Glu

2618      2628      2638      2648      2658      2668      2678      2688      2698
AAA GAT GTT GAA ACC ATT TAT ATT CCC GAC AAT GAG TCA AAA TGG GCC AGT GAA TCG ATA ATA TGT AAT GGG CAT GTT GTG CAA AAG CAA
Lys Asp Val Glu Thr Ile Tyr Ile Pro Asp Asn Glu Ser Lys Trp Ala Ser Glu Ser Ile Ile Cys Asn Gly His Val Val Gln Lys Gln

2708      2718      2728      2738      2748      2758      2768      2778      2788
AAT TTT TAT GAT TTT AGA AAC TTT TAT TAC AGT TTC ACG TAT GGA CAC TTA CAC TCA ATA CCA GAA TTT TTA GGG TCA TCT ATG ATT TAT
Asn Phe Tyr Asp Phe Arg Asn Phe Tyr Tyr Ser Phe Thr Tyr Gly His Leu His Ser Ile Pro Glu Phe Leu Gly Ser Ser Met Ile Tyr

2798      2808      2818      2828      2838      2848      2858      2868      2878
TAT GAA TAC GAT TTA AGA AAA GGA ACC AAA TCA CAT GTG TTT TTG GAT CGA ATC GAT ACG AAA AGG CTA GAG AGG AGT CTT GAC ACT TCT
Tyr Glu Tyr Asp Leu Arg Lys Gly Thr Lys Ser His Val Phe Leu Asp Arg Ile Asp Thr Lys Arg Thr Lys Arg Thr Lys Arg Thr Ser

2888      2898      2908      2918      2928      2938      2948      2958      2968
TCC TAT GGC AAT GAT AAT ATG GCA GCA ACC AAT AAA AAT ATT GCG ATC TTA ATT GAT GAC ACC ATA ATT TTG AAA AAT AAT TTA ATG TCA
Ser Tyr Gly Asn Asp Asn Met Ala Ala Thr Asn Lys Asn Ile Ala Ile Leu Ile Asp Asp Thr Ile Ile Leu Lys Asn Asn Leu Met Ser

2978      2988      2998      3008      3018      3028      3038      3048      3058
ATG AGA TTC ATC AAA CAG ATT GAT CGC TCG TTT ACT GAG AAG GTT AGA AAA GGA CCA ATA GCA AAG ATA TAT GAT TCC TTT TTG AAC TCT
Met Arg Phe Ile Lys Gln Ile Asp Arg Ser Phe Thr Glu Lys Val Arg Lys Gly Gln Ile Ala Lys Ile Tyr Asp Ser Phe Leu Thr Ser

3068      3078      3088      3098      3108      3118      3128      3138      3148
GTG AGG TTG AAT TTT TTG AAG AAT TAT TCA GTT GAA GTA TTG TGT GAA TTT TTA GTA GCG TTG AAC TTT TCA ATC CGT AAT ATT TCT TCT
Val Arg Leu Asn Phe Leu Lys Asn Tyr Ser Val Glu Val Leu Cys Glu Phe Leu Val Ala Leu Asn Phe Ser Ile Arg Asn Ile Ser Ser

3158      3168      3178      3188      3198      3208      3218      3228      3238
TTA TAC CTA GAA GAA GAA AGT GAT TGC TCC CAA AGA ATG AAT TCT CCA GAG CTG CCA AGG ATC CAC CTG AAT AAT CAA GCG CTT TCT GTC
Leu Tyr Val Glu Glu Glu Ser Asp Cys Ser Gln Arg Met Asn Ser Pro Glu Leu Pro Arg Ile His Leu Asn Asn Gln Ala Leu Ser Val

3248      3258      3268      3278      3288      3298      3308      3318      3328
TTC AAT TTA CAA GGC TAT TAC TAT TGC TTC ATC CTA ATT ATC AAA TTT TTA TTG GAT TTT GAA GCA ACT CCA AAT TTT AAG TTA CTG AGA
Phe Asn Leu Gln Glu Tyr Tyr Tyr Cys Phe Ile Leu Ile Ile Lys Phe Leu Leu Asp Phe Glu Ala Thr Pro Asn Phe Lys Leu Leu Arg

3338      3348      3358      3368      3378      3388      3398      3408      3418
ATT TTT ATT GAG TTG AGA AGC CTT CGG AAT TCT ATT TTA CTT CCC ACA CTT TCA AGA TTG TAT CCG CAA GAG TTT TCT GCA TTT CCT CAT
Ile Phe Ile Glu Leu Arg Ser Leu Ala Asn Ser Ile Leu Leu Pro Thr Leu Ser Arg Thr Leu Tyr Pro Gln Glu Phe Ser Gly Phe Pro Asp

3428      3438      3448      3458      3468      3478      3488      3498      3508
GTT GTA TTT ACG CAA CAA TTT ATA AAT AAA GAT AAT GGT ATG CTT GTC CCT GGT TTA TCC GCA AAT GAA CAC CAT AAT GGT GCA AGT GCA
Val Val Phe Thr Gln Gln Phe Ile Asn Lys Asp Asn Gly Met Leu Val Pro Gly Leu Ser Ala Asn Glu His His Asn Gly Ala Ser Ala

3518      3528      3538      3548      3558      3568      3578      3588      3598
GCT GTT AAG ACT AAG TTA GCC AAA AAG ATC AAT GTT GAA GGG CTT GCA ATG TTT ATT AAT GAA ATC CTA GTT AAC TCT TTT AAC GAT ACC
Ala Val Lys Thr Lys Leu Ala Lys Lys Ile Asn Val Glu Gly Leu Ala Met Phe Ile Asn Glu Phe Ile Leu Val Asn Ser Phe Asn Asp Thr

3608      3618      3628      3638      3648      3658      3668      3678      3688
TCT TTT TTG AAT ATG GAG GAT CCT ATT CGA AAT GAA TTT TCC TTT GAT AAT GGG GAC AGG GCA GTG ACA GAC TTG CCT CGT TCA GCA CAT
Ser Phe Leu Asn Met Glu Asp Pro Ile Arg Asn Glu Phe Ser Phe Asp Asn Gly Asp Arg Ala Val Thr Asp Leu Pro Arg Ser Ala His

3698      3708      3718      3728      3738      3748      3758      3768      3778
TTC CTA TCG GAT ACC GGC CTA GAA GGT ATT AAC TTC AGC GGC TTA AAT GAT TCG CAT CAA ACT GTT TCT ACT TTG AAT CTT TTA CGT TAC
Phe Leu Ser Asp Thr Gly Leu Glu Gly Ile Asn Phe Ser Gly Leu Asn Asp Ser His Gln Thr Val Ser Thr Leu Asn Leu Leu Arg Tyr

3788      3798      3808      3818      3828      3838      3848      3858      3868
GGG GAA AAT CAT TCA TCA AAA CAT AAA AAT GGT GGA AAG GGG CAA GGA TTT GCC GAA AAG TAC CAA TTA TCT CTG AAA TAT GTT ACT ATT
Gly Glu Asn His Ser Ser Lys His Lys Asn Gly Gly Lys Gly Gln Gly Phe Ala Glu Lys Tyr Gln Leu Ser Leu Lys Tyr Val Thr Ile

3878      3888      3898      3908      3918      3928      3938      3948      3958
GCC AAG TTA TTT TTC ACC AAT GTT AAA GAA AAC TAC ATT CAT TGT CAC ATG TTA GAT AAG ATG GCA AGT GAT TTC CAC ACT TTG GAA AAT
Ala Lys Leu Phe Phe Thr Asn Val Lys Glu Asn Tyr Ile His Cys His Met Leu Asp Lys Met Ala Ser Asp Phe His Thr Leu Glu Asn

3968      3978      3988      3998      4008      4018      4028      4038      4048
CAT CTA AAG GGA AAC AGT TGACGATTATGTCTGTCGTGATATGTGTCGCCGACTCAACTCTTTCTGATGAGATTTTGAATATCTCATTTTATTTCTCATCAACAGCC
His Leu Lys Gly Asn Ser

4078      4088      4098      4108      4118      4128      4138      4148      4158
ATGCATCGCAGTGAAGTGGACTACTGCGGGAGAACACCGCATCTATCTTTGAAATTTGTCACAAAAATATTTCACTACCTTGGCGTTTCTCATCTCAACCATTTGGGGTGAATCTCGTTA
4198      4208      4218      4228      4238      4248      4258      4268      4278
TTGCTCCTTATTTTGGTCCATTTTATATAAATCCTGTATCATGTGTCAACATTTTTCATCTTATACCTCATACAAAAAGAAAGCTATTTATGAAAGTTAGTTCATTACAAGATGCAAG
4318      4328      4338      4348      4358      4368      4378      4388      4398
CCTAAATATATGCTTGATCTTAAATTCATGTATAGAAGAAAAAGACCTTACCAACGTTGTTGGTTACCGGTATACCAATTAACCACTTGAACATTAAACCACTCTGGCGTGTGGGA
4438      4448      4458      4468      4478      4488      4498      4508      4518
GGATGTTCTTAGACTTAATTAAGAACTCTAAATTTTTTTTATTTAATCGTCCCTTTCTCAATATGAGTTTATATATTTTATAATTTCACTCAACCTCAGAAATAGCTGTGTATA
4558      4568      4578      4588      4598      4608      4618      4628      4638
TATCATTTGTCGTAATATCATCGTGAAGAACGATGCTCGTTAATTATGTCTGAATTAGCCATTCCTTAGATTGAGTGTGAATATGTAATTTAAATTTCTAAATTTCAAGTATATTT
4678      4688      4698      4708      4718      4728      4738      4748      4758
GACTTCTCAATCTTTGAGAGAGCTTCATCTGAGATTTTACCATTATTTTCGTAGCATATATGAGAACTTTAACTTTTCGATTAGATAATTCGATCTATTTCTGGGTACTAAAGATCTA
4798      4808      4818      4828      4838      4848      4858      4868      4878
AGGCATTTAAACATGGCTCTGATCATCTACATCAATATTGGGTAATCAATTGATTCATCTACCTGGCTCAACATGCAAAAGCAAAATTTGACAAAATGTCACCATCAGATCTAC
4918      4928      4938      4948      4958      4968      4978      4988      4998
CCCAACAATAACATGTAGTCATTTAACTTTTCTTCACTCCCATATCTGATCTCTTAAATTAGATCCGCGTGAAGAAATGTTGATAAAATGAAAAATATTTGTTGATTTAATGATTC
5038      5048      5058      5068      5078      5088      5098      5108      5118
CTCCCTTTGATGGAGAACTCAACGATAACCGAAAACCTTACCAGGAAGTAATAGGTATTGGTATATTAAATGTTGACTATTTCTTTCTTTCAATGCATGCATTTATGAATTTCCAGT
5158      5168      5178      5188      5198      5208      5218      5228      5238
GTAGTGTAGGCCACCCCAACGCCAAGTTTCTAAGTACTTTTAAATGCTTTTAAATGCGGTTTGTGATTAGGCAAGCAAACTCGCAAGTGTGCAATAGGGGAATGAGGTTCCAGGCTAGCTT
5278      5288      5298      5308      5318      5328      5338      5348      5358
CAAGGCCAATTCGAAATATATCTGTATTACCTTTTATGACTAAGATTTAGCTAGCGGATGAGTGAAGTAAATAAAGTGGAAAACTCTGACTCAATACAGTGGCCCTTCGAGTTC
5398      5408      5418      5428      5438      5448      5458      5468      5478
AATGGTCTGTGCAAGTTCCTCTATTTTTCGAATAGGCTGTGCAAAACAAAAATACATTTATCAATACATTCCTGTTCTGATTTCCACGAGAAAAAAGCAGGCTTGGCGCTCTGCG
5518      5528
ATGTTCAAGTAATGCGTCTCTAGA

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Table 1 Codon usage in *ADR1*

Phe TTT 49	Ser TCT* 41	Tyr TAT 34	Cys TGT* 14
TTC* 26	TCC* 24	TAC* 16	TGC 8
Leu TTA 47	TCA 38	och TAA 0	opa TGA 1
TTG* 49	TCG 17	amb TAG 0	Trp TGG 4
Leu CTT 18	Pro CCT 14	His CAT 28	Arg CGT 6
CTC 2	CCC 10	CAC* 9	CGC 6
CTA 16	CCA* 20	Gln CAA* 31	CGA 5
CTG 14	CCG 4	CAG 6	CGG 1
Ile ATT* 37	Thr ACT* 27	Asn AAT 83	Ser AGT 21
ATC* 18	ACC* 17	AAC* 36	AGC 11
ATA 20	ACA 14	Lys AAA 51	Arg AGA* 27
Met ATG 29	ACG 14	AAG* 34	AGG 16
Val GTT* 23	Ala GCT* 18	Asp GAT 63	Gly GGT* 13
GTC* 7	GCC* 11	GAC 23	GGC 10
GTA 9	GCA 22	Glu GAA* 53	GGA 10
GTG 9	GCG 9	GAG 22	GGG 12

och, ochre codon; amb, amber codon; opa, opal codon.

* Codons preferred by highly expressed genes in yeast¹⁷.

region that is rich in lysine + arginine (up to 40% per protein); this region of TFIIIA is thought to interact with other transcription factors or with RNA polymerase III as loss of this region reduced activation of transcription but did not affect DNA binding²⁴. A mutant allele of *ADR1*, *ADR1-5^c*, which confers partial constitutivity on *ADH2* expression, is located in this basic region (C. Denis, personal communication) but the reason for its effect is unknown.

An elegant model of TFIIIA structure has been proposed which attempts to account for the passage of RNA polymerase III through an internal promoter containing a stable transcription complex⁹. Each 30-amino-acid domain is proposed to form a loop or finger of ~12 amino acids. The base of the loop is formed by a Zn²⁺ bound by the cysteine and histidine residues which flank the 12 amino acids. The amino acids in the finger are suggested to bind DNA, with each finger interacting with one half-turn of the helix so that all nine fingers would be in contact with the internal control region of 5S DNA. The conserved hydrophobic residues (Tyr 6, Phe 17, Leu 23) might form a structural core of the multi-domain protein. The multiple independent binding domains of TFIIIA are postulated to allow passage of RNA polymerase III through the internal control region without dislodging the stable transcription complex.

The importance of the finger-like domains of *ADR1* (Fig. 2) for activating *ADH2* expression is suggested by experimental data in addition to its homology with TFIIIA. Previous experiments indicated that a 2.6-kilobase (kb) *ADR1* fragment allowed partial derepression of *ADH2* in a strain containing the *adr1-1* allele⁵. The sequence data presented in Fig. 1 demonstrate that this fragment of *ADR1* would encode only the amino-terminal 302 amino acids of the protein. To determine which region of the *ADR1* protein is required for *ADH2* derepression, a series of 3'-deleted *ADR1* genes was constructed in the yeast/*Escherichia coli* shuttle vector pMW5 (Table 2). The *ADR1* plasmids were introduced into strain 521-6-Δ1 in which the 5'-flanking region and 2 kb of coding sequence of *ADR1* have been replaced by the *LEU2* gene of yeast (data not shown). In this strain *ADR1* structure/function relationships can be assessed in an *adr1*-null genetic background which eliminates the possibility of intragenic complementation between two defective *ADR1* genes.

As shown in Table 2, an amino-terminal 150-amino-acid *ADR1* peptide is unable to derepress *ADH2*. A 302-amino-acid *ADR1* peptide can derepress *ADH2* but is only 20% as active as the entire protein. *ADR1* proteins containing the amino-terminal 505 amino acids are as active as the entire protein. These results suggest that a domain required for *ADR1* function exists between amino acids 150 and 302 and that sequences located between amino acids 302 and 505 may be required for full *ADR1* activity. Therefore, most of the *ADR1* protein (amino

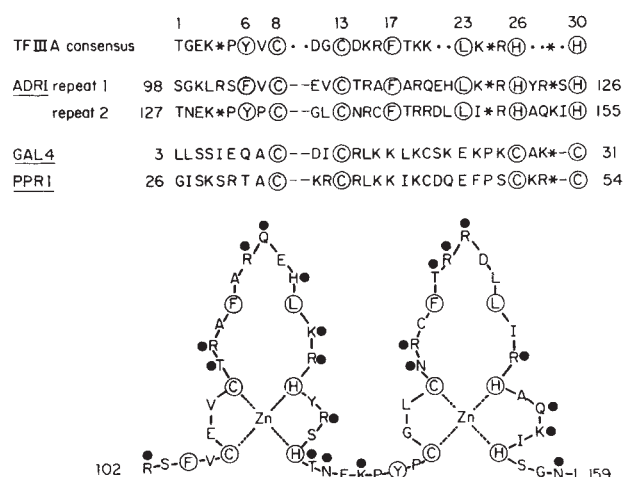


Fig. 2 The amino-acid sequences of the TFIIIA 30-residue repeat compared with amino-acid sequences of yeast regulatory proteins and the proposed 'finger' structure of *ADR1*. The TFIIIA consensus sequence was compiled by Miller *et al.*⁹ from the nine contiguous repeat units in the TFIIIA protein. The invariant residues are circled. The numbers above the TFIIIA sequence refer to the amino-acid position in the consensus sequence, not in the TFIIIA polypeptide chain. The numbers adjacent to the other sequences refer to the position of the amino acid in the polypeptide chain. In the consensus sequence, dots mark variable positions in the sequence, and asterisks indicate where insertions sometimes occur in the normal pattern. In the other sequences, a dash (-) indicates a gap introduced for alignment. Each repeat is postulated to form a finger-like structure wherein the two cysteine (C) and two histidine (H) residues bind a Zn²⁺ in a tetrahedral arrangement⁹. The hydrophobic residues tyrosine (T), phenylalanine (F) and leucine (L), are proposed to form a structural core for the multiple domains⁹. The region between the second cysteine and the first histidine residue may form a DNA-binding loop. TFIIIA repeat number 7 (ref. 9) has the same gap alignment between its cysteine residues as the *ADR1* repeats. Bottom, the proposed finger structures of *ADR1*, drawn according to the model proposed by Miller *et al.*⁹. Residues with probable DNA-binding side chains [lysine (K), histidine (H), glutamine (Q), asparagine (N), threonine (T) and arginine (R)] are indicated by solid circles.

acids 505–1,323) is not required for its role in *ADH2* transcriptional activation.

The shortest *ADR1* protein that retained *ADH2*-stimulating activity (containing 302 amino acids) contained the finger region as well as the adjacent Lys + Arg-rich region. The next smaller *ADR1* protein (containing 150 amino acids) was inactive; it lacked the very basic region and retained only the first finger and one-half of the second. Thus a single finger alone may not be sufficient to derepress *ADH2* expression, but whether this is due to loss of the second finger, loss of the very basic region, or both, cannot be distinguished at present. Additional genetic evidence that this finger region of *ADR1* is functionally important derives from DNA sequence analysis of mutants of *ADR1* (C. Denis, personal communication). Two mutants which derepress *ADH2* very poorly contain single amino-acid changes in the finger region of *ADR1*. Neither mutation alters an invariant residue of the TFIIIA consensus sequence. These alterations provide strong evidence, consistent with the deletion analysis reported here, that the finger region is essential for *ADR1* function. By analogy with the proposed structure of TFIIIA, this region would be required for DNA binding. The analysis suggests that one DNA-binding finger is insufficient for *ADR1* activity.

It seemed unlikely that *ADR1* would be the only yeast protein with structural homologies to TFIIIA. The nucleotide sequences of several yeast regulatory proteins have been reported recently^{13,14}, including the proteins encoded by *GAL4* and *PPR1* which contain a basic, cysteine-rich amino terminus (Fig. 2). In

Table 2 Functional characterization of *ADR1* genes containing variable-length 3'-terminal deletions

Relevant yeast genotype	Plasmid	ADH activity (<i>dr</i>)
<i>adr1-Δ1</i>	pMW5	25
<i>adr1-Δ1</i>	YEp150ADR1	20
<i>adr1-Δ1</i>	YEp302ADR1	1,400
<i>adr1-Δ1</i>	YEp505ADR1	6,500
<i>adr1-Δ1</i>	YEp1323ADR1	6,500
<i>ADR1</i>	pMW5	2,000

Plasmid constructions: All constructions used the yeast *E. coli* shuttle vector pMW5, which is the same as plasmid MA56 but lacks the *ADHI* promoter fragment³¹. This plasmid contains the 2μ origin of replication and *TRP1* gene for selection in yeast, as well as the pBR322 sequences necessary for growth and ampicillin-resistance selection in *E. coli*. Plasmid DNA was prepared by the method of Birnboim and Doly³² and *E. coli* strain RR1 was used for transformation. The restriction enzyme digests of pMW5 and *ADR1* plasmids used to construct the series of 3'-deleted *ADR1* genes are described below. To construct YEp150ADR1 and YEp505ADR1, p411-B (which contains a 5-kb *Bam*HI fragment from clone YRp7-*ADR1*-411)⁵ was digested with *Bam*HI plus *Sph*I (YEp150ADR1), or *Bam*HI plus *Nru*I (YEp505ADR1). To construct YEp302ADR1 and YEp505ADR1: *Bam*HI plus *Pvu*II; YEp150ADR1: *Bam*HI plus *Sph*I; YEp1323ADR1: *Pvu*II. Yeast transformation: Yeast transformations were done using the lithium acetate procedure³³ on 521-6 (*mata adh1-11 ADH2 adh3 ADR1 trp1 ura1 leu2*) and 521-6-Δ1 (*mata adh1-11 ADH2 adh3 adr1-Δ1::LEU2 trp1 ura1 leu2*). ADH assays were performed as described previously⁵. Derepressed cultures (*dr*) were grown in yeast complete medium containing either 3% ethanol or 1% ethanol plus 3% glycerol. Assays were done in triplicate for at least two independent transformants. The values are the means for six assays. Derepressed values in strain 521-6-Δ1 carrying YEp1323ADR1 were corrected for the number of cells that had lost plasmid since plasmid loss occurred at a higher frequency in these strains than in the other transformants.

this region, two pairs of cysteines are separated by 13 amino acids, similar to *ADR1* and *TFIIIA* repeat sequences. Thus, a finger of similar size could be formed with four cysteines acting as ligands for Zn^{2+} rather than two cysteine and two histidine residues.

The evidence that a common structure could exist in transcription factors from such divergent species as yeast and frog, which are active with different RNA polymerases, suggested that similar structures might exist in other proteins with DNA-binding activity. Several complementary DNA clones and genes from *D. melanogaster* have recently been found to have this same repeated sequence motif (refs 11, 12 and J. Lengyel, personal communication). One of these genes is derived from *Krüppel*^{11,26}, a locus affecting early *Drosophila* development; several of the others are either derived from or are adjacent to blastoderm-specific genes. All these *Drosophila* genes might thus have roles that are important in development, for example as tissue- or gene-specific transcription factors.

The best-characterized protein structure which is involved in DNA binding is the helix-turn-helix motif found in a variety of prokaryotic regulatory proteins²⁷, in the yeast *MATα2* repressor^{28,29} and in the homeo box region of several eukaryotic proteins³⁰. The data presented here suggest that there may be a second structural motif of DNA-binding proteins in eukaryotes typified by the 30-amino-acid repeat found in *TFIIIA* and *ADR1*. The structural homology between *ADR1* and *TFIIIA* suggests that the factors regulating transcription by the different RNA polymerases have been under strong selective pressure to maintain basic structures and functions required for interaction with RNA polymerase and DNA.

We thank Dr Russell Doolittle for his computer-aided analysis of the amino-acid sequence of *ADR1* which revealed the homology between *ADR1* and *TFIIIA*; Dr Clyde Denis for his communication of the nucleotide changes in *ADR1* mutants; Dr Jeff Shuster for pointing out to us the potential phosphorylation sites in *ADR1*; Dr Richard Palmiter for critical reading of the manuscript; and Kate Roudybush for careful preparation of the manuscript. Support for this research was provided by the USPHS (GM26079) and PHS NRSA 5 T32 GM07270 from NIGM.

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Eukaryotic ribosomes that lack a 5.8S RNA

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The 5.8S ribosomal RNA is believed to be a universal eukaryotic characteristic¹. It has no (size) counterpart among the prokaryotes, although its sequence is homologous with the first 150 or so nucleotides of the prokaryotic large subunit (23S) ribosomal RNA. We report here an exception to this rule. The microsporidian *Vairimorpha necatrix* is a eukaryote that has no 5.8S rRNA. As in the prokaryotes, it has a single large subunit rRNA, whose 5' region corresponds to the 5.8S rRNA.

Microsporidia, of which *V. necatrix* is representative, are parasitic eukaryotes with various interesting life cycles². Microsporidian species infect virtually all types of animals, from invertebrates to man³. Their ribosomes and rRNAs have been reported to be prokaryotic in size; the ribosome is a 70S (not 80S) particle that comprises 30S and 50S (not 40S and 60S) subunits, which in turn contain 16S and 23S rRNAs^{4,5}. While isolating *V. necatrix* RNAs, we observed not only these unusual prokaryotic electrophoretic mobilities, but an unexpected absence of a 5.8S RNA. This fact can be explained in several


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pos.(001-100)
E. coli      GGUUAAGCGA CUAAGCGUAC ACGGUGGAUG CCCUGGCAGU CAGAGGCGAU GAAGGACGUG CUAUUCUGCG AUAAGCGUGG GUAAGGUGAU AUGAACCGUU
V. necatrix  ----- -nnnaCCCAC aCaUGGGAUC aAUaGGAUAC CA-UaACgAU GAAGGUCGUa AUaGAAUACG A-AAGUAUUAU ----- UAUUUaCCUG
S. cerevisiae ----- -AAACUUUCA ACAACGGAUC UCUUGGUUUC CGC-AUCGAU GAAGAACGCA GCGAAAUCCG AUACGUAUUG UGAUUUGCAG --AAUUCGU

pos.(101-180)
E. coli      AUAACCGGCG AUUUCGGAU GGGGAAACCC AGUGUGU--UUC- -GACACACUAU CAUUAACUGA AUC-----CAUAGGU UAAUGAGGCG
V. necatrix  -AUUAAUAUA UUUaUaA--- --UGUAUUGA U-----CAUAGGU UAAUGAGGCG
S. cerevisiae GAUUAUUGA AUCUUUGAAC GCACAU-UGC GCCCUUGGUUUAU CCAGGGGGCAU GCCUGUUUGA GCGUAUUUxxUUGACCUCAAAU CAGGUAGGAG

pos.(181-250)
E. coli      AACCGGGGGA ACUGAAACAU CUAAGUACCC CGAGGAAAAG AAAUCAACCG AGAUUCCCCC AGUAGCGGCG
V. necatrix  uACCCUUUGA ACUUAAGCAU AUCuUUnAAA GGAGGAAAAG AAACUuACuU GGAUUCUUUU aGUAGCAGCG
S. cerevisiae UACCCGCGUA ACUUAAGCAU AUCAAUAAAG GGAGGAAAAG AAACCAACCG GGAUUGCCUU AGUACGGGCG

```

Fig. 1 Sequence alignment for the initial portion of the 23S-like ribosomal RNAs from *Escherichia coli*⁹, *Saccharomyces cerevisiae*¹⁰ and *Vairimorpha necatrix*. The alignment procedure was that used in ref. 8. Numbering is according to the *E. coli* 23S rRNA sequence. Positions where composition is uncertain are shown in lower case, using 'n' if the composition is undetermined. Dashes (inserted for alignment purposes) signify gaps in a given sequence. The join between the yeast 5.8S and 25S rRNAs is indicated by 'xx'.

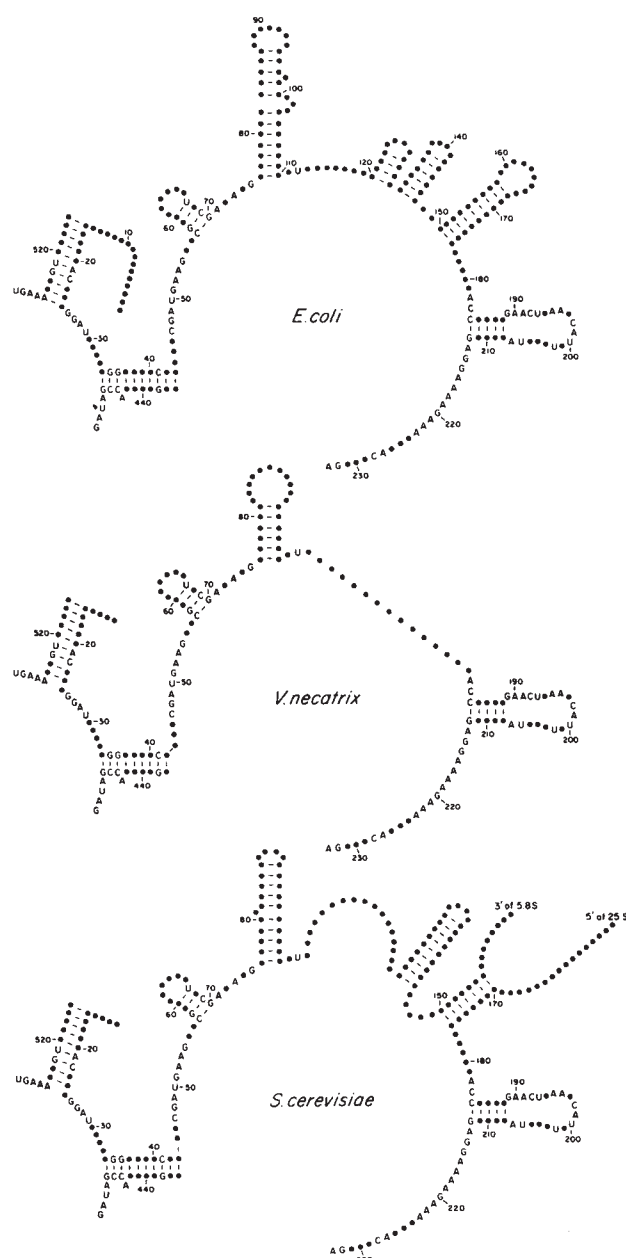


Fig. 2 Secondary structure comparisons for the initial portion of the 23S-like rRNAs from *E. coli*⁹, *S. cerevisiae*¹⁰ and *V. necatrix*. Secondary structure is taken from ref. 8. All positions in the sequences are indicated by dots except for those in which secondary structural elements are homologous and composition is common in all three cases. Position numbers refer to the *E. coli* 23S rRNA sequence.

ways. To decide among them, we sequenced the appropriate portion of the 23S rRNA of the organism.

Several regions in the 5' half of the *V. necatrix* 23S rRNA were sequenced using reverse transcriptase, employing primers complementary to the more highly conserved areas in the rRNA sequence^{6,7}. Figure 1 shows the sequence of the 5' 200 or so nucleotides aligned with its counterparts from *Escherichia coli* and *Saccharomyces cerevisiae*⁸⁻¹¹. Secondary structural representations of the three are shown in Fig. 2. Primary and secondary structural homology is apparent among all three sequences both before and after the join between the eukaryotic (yeast) 5.8S RNA and the 25S rRNA. The helix formed between the 3' 10 or so nucleotides of the yeast 5.8S RNA and the 5' 10 or so nucleotides of its 25S rRNA is completely missing in *V. necatrix* (Fig. 2). The *V. necatrix* 23S rRNA has its 5' end at about the same position (relative to *E. coli*) as does the yeast 5.8S RNA, and the former continues in an unbroken fashion thereafter.

It is unwise to draw any but the most general phylogenetic conclusions from the *V. necatrix* sequence, because this particular area of the 23S rRNA varies in structure, and the lack of structural homology greatly reduces the phylogenetic value of much of the sequence. This segment of the *V. necatrix* rRNA is closer to its yeast counterpart than to the *E. coli* sequence, while the *E. coli* sequence is in turn closer to the yeast sequence than to that of *V. necatrix*. A more extensive study, based on 16S rRNA, is now in progress (C.R.V., unpublished), and shows that *V. necatrix* clusters specifically with the other eukaryotes, but forms a remarkably deep branching in the common line of eukaryotic descent.

Our results do not indicate whether the single continuous 23S rRNA condition seen in the microsporidian is an evolutionary forerunner to the split '23S' seen among the other eukaryotes, or represents fusion of ancestral 5.8S and 25S rRNAs. We favour the former interpretation, because the covalently linked condition is characteristic of both prokaryotic kingdoms, and *V. necatrix* does branch deeply in the common line of eukaryotic descent.

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Agglutination methods for rapid analysis

from Mel Thomas

Tests making use of carrier-bound antibodies can identify bacteria in less than five minutes, with results visible to the naked eye.

USING one of the first microscopes, Antony Van Leeuwenhoek observed and described 'animalcules' in 1676. His drawings record the chief forms of bacteria — cocci, rods, filaments and spirochaetes. Hence Van Leeuwenhoek has been dubbed the 'father of bacteriology'.

More than two hundred years later, Robert Koch developed solid nutrient media and staining methods that made growth and observation of these bacteria much easier¹. These fundamental discoveries spawned a system for bacterial isolation and identification which is still in use today. It involves growing bacteria in primary culture, subculturing onto varied specialized media and observing patterns of substrate utilization. These biochemical identification tests are reliable and specific, but take at least two days and sometimes several weeks to provide results. The need for more rapid and less laborious identification methods led to the development of coagglutination and latex agglutination testing.

In 1973, Göran Kronvall at the University of Lund, Sweden, reported a unique method for typing *Streptococcus pneumoniae* which was not only rapid, but also very easy to perform². Kronvall called his method 'coagglutination'. Later that same year Christensen *et al.* described the use of coagglutination for the serological grouping of streptococci³. This research demonstrated that pathogens from overnight cultures could be identified in minutes instead of days.

The lattice link

Coagglutination is an immunodiagnostic technology that utilizes antibodies in a reagent to detect specific bacterial antigens. The reagent consists of a suspension of non-viable staphylococci onto which specific antibodies are adsorbed. Recently the same principle has also been applied to polystyrene particles as carriers for latex agglutination. When a sample containing the homologous antigen is mixed with the reagent, an antigen-antibody linkage occurs which forms a lattice visible to the naked eye.

Pharmacia Diagnostics AB obtained commercial rights to the technique and began marketing its own improved pathogen identification tests in 1975. Now test kits can diagnose streptococci groups A, B, C, D, F and G, gonococci, meningococci, pneumococci and encapsulated types of *Haemophilus*.

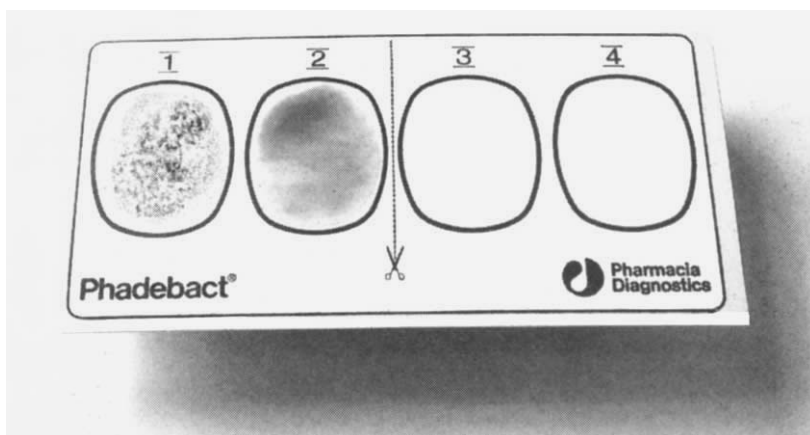


Fig.1 If a sample tests positive, a fine precipitate visible to the naked eye forms from the lattice linkage of antibodies and antigens.

The original method for the preparation of coagglutination reagents has been published³. *Staphylococcus aureus* (strain Cowan I) is grown overnight in tryptic soy broth kept at 35°C and aerated. The harvested bacteria are washed twice with phosphate-buffered saline (PBS: 0.15 M NaCl, 0.01 M phosphate, pH 7.4), and suspended in 0.5 per cent formaldehyde in PBS at room temperature for 3 hours. The formaldehyde-treated suspension is then washed four times in PBS, and adjusted to a final concentration of 10 per cent (v/v).

Protein A, which binds IgG class antibodies to staphylococcus cells by their Fc fragments only, will be stable for several weeks using the above protocol. Heat treatment at 80°C for 2 hours kills the bacteria and extends stability to months if the reagent is stored at 4°C. Finally, the formaldehyde- and heat-treated staphylococci are mixed with a specific antiserum. After washing and resuspension in PBS containing 0.1 per cent sodium azide, the reagent is ready for testing.

To perform the test, a drop of reagent is added to a white slide on which either a smear from a primary culture, or a direct sample, has been placed. The slide is rocked gently back and forth as the reaction proceeds. If the result is positive, a fine precipitate will form within 2 minutes (see Fig.1).

A latex alternative

Latex agglutination is similar to coagglutination in that it employs a carrier, latex, to which specific antibodies are adsorbed. The preparation of latex reagents is also well-documented⁴. One part of 0.81 µm polystyrene latex particles is combined

with one part antiserum which has been diluted to an empirically defined optimal reactivity concentration. The suspension is incubated at 37°C for 2 hours. Then, two volumes of glycine-buffered saline (1.0 M glycine and 1 per cent NaCl, pH 8.2) containing 0.1 per cent bovine serum albumin is added. The sensitized latex reagent is stored at 4°C.

Antibody adsorption on staphylococci and latex particles is quite different. Protein A, present on treated staphylococci but not on latex, orients the antibodies so that the Fab structures are open for reaction; latex, however, adsorbs antibodies randomly, as shown in Fig. 2. Hence a portion of the antibodies adsorbed on latex are rendered useless for immunodiagnostic purposes. This does not mean that latex agglutination is a less sensitive technique than coagglutination. It does mean, however, that different antibody concentrations may be needed, as well as some form of sample preparation, for instance an extraction process.

Collective benefits

The advantages of coagglutination and latex agglutination are many. Because testing a primary culture takes less than 5 minutes, specific antibacterial therapy can be initiated at the earliest possible moment, perhaps preventing morbidity and mortality due to infection. Agglutination methods do not require living organisms for testing, and so avoid the problems of bacterial identification associated with live cultures.

Direct testing is also possible on certain specimens using coagglutination or latex agglutination. Cerebrospinal fluid, serum

and urine can be used directly in tests for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus* group B. Recently, agglutination tests for the identification of *S. pyogenes* (*Streptococcus* group A) directly from throat swabs have been introduced. In this case, rapid bacterial identification has a real benefit in that it protects patients who do not have group A streptococcal pharyngitis from the needless use of antibiotics.

The simplicity of coagglutination and latex agglutination testing cannot be over-emphasized. Testing is done by hand,

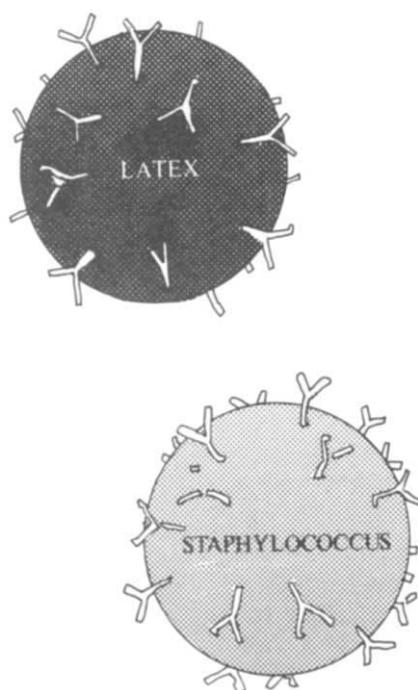


Fig. 2 Antibodies adsorb randomly to latex, but the protein A coating staphylococci anchors antibodies by their Fc fragments.

without the need for expensive and complicated equipment. No special training is required, and the final cost of bacterial identification can be much less than that of prolonged biochemical procedures.

What of the future? Agglutination technology is taking advantage of highly specific monoclonal antibodies, and tests are being developed for viruses as well as additional bacteria. The areas of development are limited only by the levels of sensitivity that can be achieved. That which was started by Van Leeuwenhoek and Koch has gained new impetus from Kronvall and his successors. □

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Taking a closer look at microbiology

Bacteria, fungi and yeast have captured a degree of attention that belies their dimensions. This week's review examines microbial techniques from the fermenter to the autoclave.

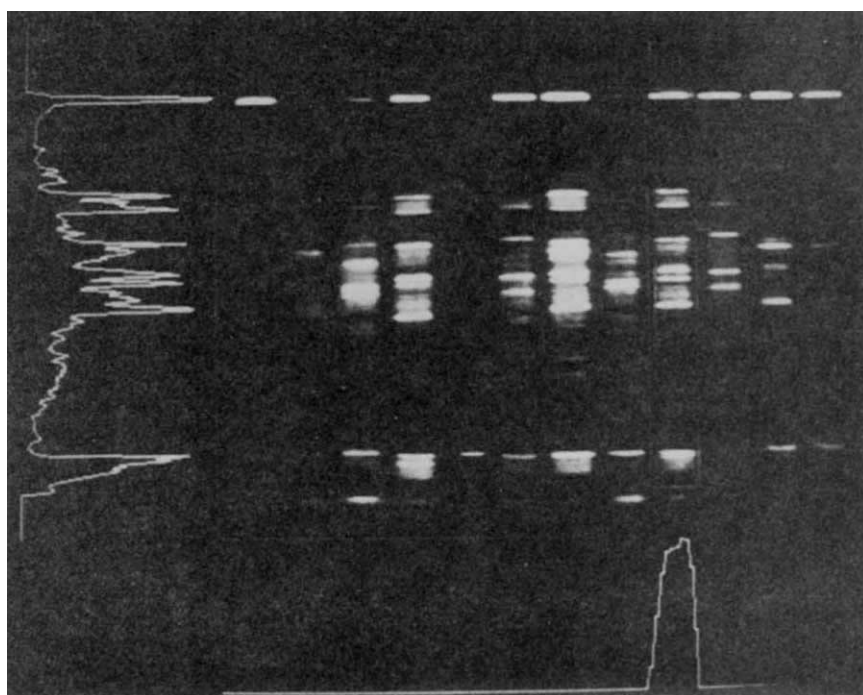
A NON-RADIOACTIVE DNA probe to rival avidin-biotin methods has emerged from the laboratories of Biotech Research (Reader Service No. 100). Beginning in May, Biotech will offer testing kits for viral and bacterial infection based on **hapten-antibody binding**. Because the system is chemical rather than enzymatic, Biotech expects to be able to sell the kits for one-half to one-third the cost of most non-radioactive probes. The company is developing both 'do-it-yourself' kits for customizing probes, and kits for specific viral and bacterial genes.

Back on the avidin-biotin front, Enzo Biochem Inc. have refined their **DNA probe system** in Pathogene II kits that even an amateur can use (Reader Service No. 101). The non-radioactive assay kits are available for infectious agents including *Chlamydia trachomatis*, herpes simplex virus, Epstein-Barr virus, cytomegalovirus (CMV) and adenovirus 5. Step-by-step instructions and all the reagents and buffers for 20 assays come in one package, priced at \$135 (US), except for the \$155 CMV kit.

The two-dimensional **AMBIS beta scanning system** was originally created to

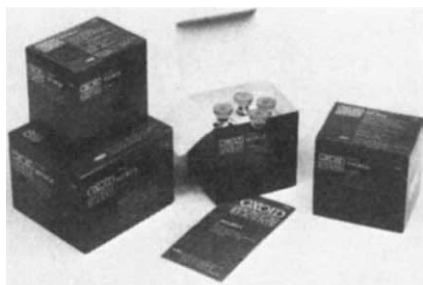
quantitate the gel patterns Robert Silman used to 'fingerprint' bacteria. When Automated Microbiology Systems Inc. set out to market the identification system, they recognized a need for a fast, convenient scanner that would display the scan in autoradiograph form. Now their AMBIS system can do that, and much more. The scanner will quantitate beta-emitting isotopes from thin-layer chromatography plates, transfer membranes and whole body sections as well as gels, in as little as 15 to 30 minutes (Reader Service No. 102). An IBM PC/AT with a 20-megabyte hard disk and Hercules graphics stores the results and makes data extraction and quantitation simple. The scanner sells for \$24,000 (US), or \$29,000 with the computer included.

A **gene identification system** for Gram-negative bacteria promises to make matching mutant phenotypes to mutated genes a little easier (Reader Service No. 103). The system relies on a transposon in the plasmid vector that tags the region at which the vector DNA became incorporated into the recipient genome, causing mutation. Allelix Inc. in Canada will license to or undertake collaborative work with interested firms or organizations.



The AMBIS system scans all lanes simultaneously to assemble a composite image.

In cases where finding a bacterial toxin is more important than detecting the bacteria itself, **toxin identification kits** may be



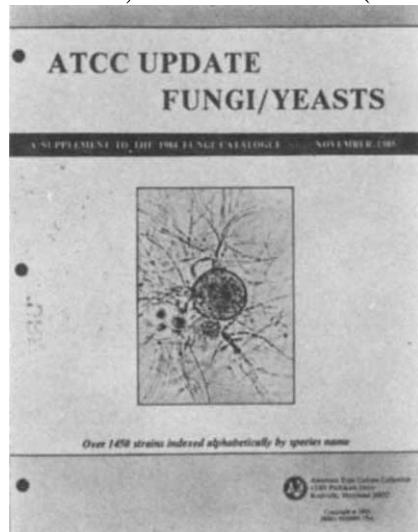
Toxin detection via latex agglutination.

the best bet. Oxoid Ltd. recently launched four such kits that employ the reversed passive latex agglutination (RPLA) technique for detection (Reader Service No. 104). Their sensitivity is as low as 1 or 2 ng of toxin per ml because the Oxoid's antibodies are highly purified. Kits are available for staphylococcal enterotoxins A, B, C and D, *Vibrio cholerae* enterotoxin/E. coli heat labile enterotoxin, *Clostridium perfringens* enterotoxin and staphylococcal toxic shock syndrome toxin. Each kit provides for 20 tests; costs vary.

Culture media formulated for the **selective isolation** of microbial species is one way to be sure what's growing where. Selectatab media for *Campylobacter* and *Yersinia* isolation are the latest additions to Mast Laboratories Ltd's media list (Reader Service No. 105). The products come in packs of 25 tablets for less than £15 each, and each tablet is suited for 250 ml of the appropriate medium. All tablets have a shelf life of two years.

Culture counts

If supplies of **filamentous fungi** and yeasts are running low, check the updated *ATCC Catalogue of Fungi/Yeasts* for more than 1,450 brand new strains (Read-



Alphabetical listings provide easy access in the ATCC's latest catalogue.

er Service No. 106). The American Type Culture Collection has added strains that are useful in biological control, degradation, production, resistance/sensitivity, testing and assays, including controls for antibiotic sensitivity tests. ATCC also provides information on special applications, genotypes, pathogenicity, recommended media and growth conditions wherever possible.

The EpiCount kit from Nucleopore performs **counts of liquid-borne microorganisms** in less than 30 minutes (Reader Service No. 107). The EpiCount procedure involves trapping microbes on a black polycarbonate membrane with a 10 µm pore size (ordinary cellulose membranes



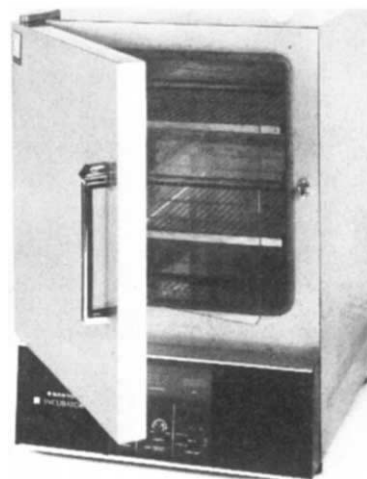
Nucleopore's epifluorescent counter uses an acridine orange stain.

have pore sizes between 150 and 200 µm). The small pore size creates a surface capture effect which, in combination with the dark background, improves the accuracy of epifluorescent counting. The essential elements for Nucleopore's system, except the microscope, come in a \$695 (US) kit.

Fluorescence activated cell sorters (FACS) can pick out a microbe in a million, and the more parameters specified, the more particular the selection. Becton Dickinson have upgraded the options on their FACS 440 dual laser bench flow cytometers to include five-parameter status (Reader Service No. 108). A new £19,000 (UK) He-Ne laser is suitable for two- and three-colour immunofluorescence for antibody marking, as well as forward and 90 degrees light scatter analysis to indicate size and granularity. The 5 watt laser provides 32 mW of power at 633 nm with a typical tube life of 20,000 hours.

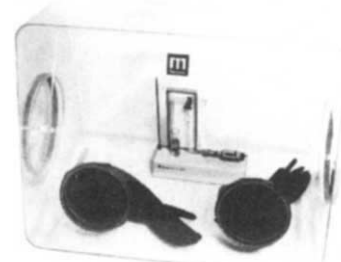
Chamber choices

Even heating and minimum deviation make Gallenkamp's **air jacket incubators** reliable laboratory companions (Reader Service No. 109) Indirect heating with the air jacket design eliminates the need for mechanical convection, while a sophisticated electronic thermostat keeps temperature steady. Gallenkamp uses adjustable



With built-in protection systems temperatures in Gallenkamp incubators never stray more than 2°. stainless steel shelves to allow flexible interior space. The incubators, available in 93 and 153 litre capacities, run £825 and £1,006 (UK) respectively, and each has a temperature range from 5 to 60°C.

Manostat Corp. presents an economical way to get into controlled environments with their new **glove box**, priced under \$1,500 (US) (Reader Service No. 110). Options available with the box include bare hand entry, air lock and a selection of

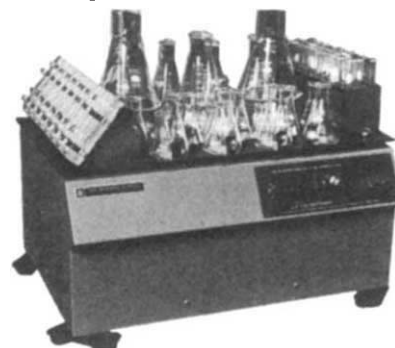


Two or more Manostat glove boxes can be connected with a coupling sleeve.

different gloves. Plexiglass construction makes customization easy for special attachments such as valves or hose ends.

Shaken and stirred

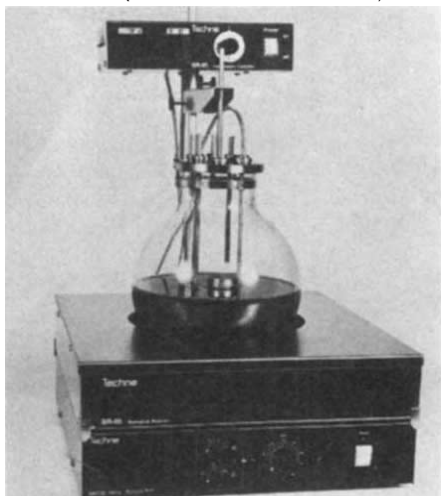
Switching flask or test tube sizes in **biological shakers** often means changing platforms as well. But New Brunswick Scientific have perfected their 'universal' plat-



A New Brunswick shaker's drive mechanism is unfazed by unbalanced or heavy loads.

form with over 200 holes, to accommodate up to 12 different sizes of Erlenmeyer flasks at one time or hold slant and test tube tracks (Reader Service No. 111). Flasks can range from 25 ml to 6 litres; test tubes, from 13 to 25 mm. The 18 × 30 inch platform is priced at \$400 (US).

By floating an impeller on top rather than using a sunken spin bar, Techné Inc. say they've improved the rate of solution of oxygen by 50 to 80 per cent in their latest **bioreactor** (Reader Service No. 112). The



The BR-05 bioreactor is all glass with a stainless steel multi-port cap.

2.5-litre BR-05 has a spherical shape that increases the ratio of surface area to volume over that of a comparable cylin-

drical vessel. That feature disperses oxygen in the bioreactor without forming air bubbles. The \$3,500 (US) unit comes with a platinum temperature sensor (to be submerged in the medium), stirrer and speed control.

An **integrated bioreactor system** from Queue Systems can perform both fermentation and cell culture with individual



Data from the integrated Queue Mouse fermenter can be sent to a printer or another computer.

control of up to 21 variables (Reader Service No. 113). Complete with pre-programmed software, the Queue Mouse works in batch, feed batch and continuous culture applications, as well as hollow fibre, mechanically agitated and airlift bioreactors. For about \$20,000 (US), the Mouse system offers flexibility as its strong point.

Computer-controlled **gradient feed technology** has enabled Battelle to produce recombinant *E. coli* cells in concentrations of over 80 grams (dry weight) per litre (Reader Service No. 114). The fermentation know-how is available from Battelle in a technical manual, a computer software package and follow-up technical services for under \$15,000 (US). The company expects that their system will also be applicable to genetically modified *Bacillus subtilis*, other bacteria and yeasts.

Applikon's series of **small and middle-range fermenters** runs the gamut of fermentation unit size and specificity (Reader Service No. 115). They offer fermenters suited for cell or continuous cultures in round bottom, flat bottom or jacketed glass vessels and stainless steel bioreactors. Each has plenty of ports on the headplate. Applikon's stirrer assemblies were designed to avoid contamination, in double lipseal or magnetic-coupled stirrer versions. Prices vary depending on the choice of unit and options.

The end of the line

When it's time to wrap up an experiment, Riese Enterprises has a combination

waste disposal/sterilization indicator bag designed for biohazardous waste (Reader Service No. 116). Bright red, melt-resistant BioSure bags integrate time, temperature and pressure to signify, by means of a migrating blue chemical, when contents have been safely autoclaved. The bags come in packs of 100 and cost \$78 (US) for the 19 × 24 inch size and \$85 for the 24 × 36 inch bags.

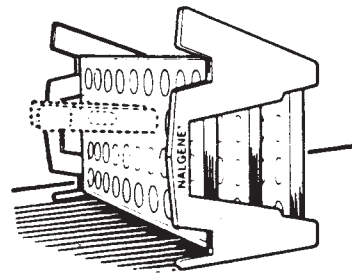
Bacteria and viruses are easy prey for Kontes' micro **ultrasonic cell disrupter** (Reader Service No. 117). The disrupter will produce rapid and reproducible results with micro- to millilitre sample volumes. Monitor circuitry maintains the displacement amplitude of the probe tip under widely varying loads, and an LED indicator displays the load imposed by the



A tiny probe from Kontes makes for small-scale cell disruption.

sample. The combination of these two features ensures reproducible figures, with a \$1,230 (US) price tag.

Nalgene's 1986 **labware catalogue** covers over 400 of their plastic lab products in 118 pages (Reader Service No. 118). The catalogue leaves no stone unturned, listing full product specifications, prices, chemical resistance/physical properties data and care and use instructions for every item. In addition to routine illustrations, this year's booklet contains a 14-page colour section featuring Nalgene's new disposable filter units. Test tube and vial



Every product gets a picture in Nalgene's new catalogue.

racks, centrifuge bottles and laboratory jars, carboys and Petri plates are offered for the microbiologist's inspection. □

These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

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